ANNUAL REVIEW OF PHYSIOLOGY

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# ANNUAL REVIEW OF PHYSIOLOGY

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VOLUME 18

1956
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FOREIGN AGENCIES

H. K. Lewis & Company, Limited
136 Gower Street, London W. C. 1

The Maruzen Company, Limited
6 Tori-Nichome, Nihonbashi
Tokyo

PRINTED AND BOUND IN THE UNITED STATES OF AMERICA
BY THE GEORGE BANTA COMPANY, INC.

# PREFACE

The present volume of the *Annual Review of Physiology* is unique in that it was planned during the Nineteenth International Congress of Physiology at Montreal. The Editorial Committee was aided by a group of Honorary Members consisting of Professors E. D. Adrian, U. S. von Euler, C. Heymans, G. Moruzzi, and A. Rosenblueth. The roster of authors chosen reflects our widened acquaintance with workers throughout the world. Thus we have three chapters from Sweden, two each from England and France, and one each from Argentina, Belgium, Canada, Germany, and the Netherlands. The remaining ten are from the United States. We were happy to hear from our Honorary Members that they felt the *Review* in past years had, in general, surveyed contributions from various countries in proper proportion to their scientific productivity. Where discrepancies existed, it was recognized that language barriers were responsible. In this, the *Review* faces a problem common to all forms of scientific communication.

It is particularly fitting that the Prefatory Chapter to this volume has been written by Dr. Bernardo Houssay, whom we recognize not only as a distinguished contributor to physiological knowledge, but also as a heroic defender of the freedom of science and advocate of its internationalization. No better statement of our aim for this publication could be made than the concluding sentence of his chapter: "The scientific fraternity should be bound by ever closer bonds, and should be an example of fraternity and peace for all mankind."

The international scope of our authorship would have been still broader if illness had not compelled Dr. L. C. Beadle of Kampala, Uganda, East Africa to postpone his chapter on the Comparative Physiology of Ionic Regulation to the next volume. Circumstances have also forced Dr. W. Mommaerts to defer appearance to next year of his chapter on the Physical Properties of Protoplasm. Otherwise our roster is complete.

Finally we again wish to acknowledge the loyal service of our Editorial Assistants, Michele Leiser, Suzanne Rebourgière, and Lillian Rutherford, whose labors have made an essential contribution to the preparation of this volume. We also wish to thank our printer, the George Banta Company, Inc., for their part in this task.

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## ERRATA

Volume 17

Page 172, reference 90 should read "Gauer, O. H., Sieker, H. O., Henry, J. P., and Wendt, W. A." We regret the unintentional omission of Dr. Wendt's name in this entry.

Page 228, line 17. For "periods of 6 to 8 hr." please read "periods of 6 to 48 hr."

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BERNARDO A. HOUSSAY, M.D., Sc.D.

# TRENDS IN PHYSIOLOGY AS SEEN FROM SOUTH AMERICA

By B. A. Houssay

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Physiology is one same science throughout the world, since the functions of living organisms are the same everywhere. The advancement of its knowledge, however, and of its applications has varied considerably in different countries. It is, therefore, useful to take into account the opinions of men who for many years have been active in teaching and research in different parts of the world. The opinions expressed here are those of a South American who entered the University in 1901 and began to do research work (hypophysectomy) In 1908; he became a university professor in 1910, and in 1919 was the first in his country to be appointed a full-time professor; since 1944 he has directed a private research laboratory.

Universities were founded in Spanish America (Santo Domingo, 1538; México and Lima, 1571; Córdoba, 1622) before the first American English-speaking University was established (Harvard, 1636), but scientific development has been much greater in the latter than in the former. In a recent paper (1) I have discussed some of the causes of this: dogmatic tendencies, the idea that universities must teach knowledge that has been definitely acquired, an exclusively professional outlook, and lack of understanding of the significance of science and research. In another paper (2) I have described the historical development of physiology in Argentina. In both these I gave reasons for being optimistic with respect to the future.

The development of physiology in Argentina is similar to that in other countries of America. The Chair of Physiology was separated from that of Anatomy in 1873. This was attributable, in great part, to the impact of Claude Bernard's work because in our country medicine was then under French influence. In 1889, the first lecture on experimental physiology, with demonstrations on animals, was delivered to a Medical Society by Dr. J. B. Señorans, who had been trained in France and England. In 1896 instruments for the Chair of Physiology were imported from Europe for the first time, and in 1899 the Italian Professor V. Grandis accepted a contract to direct practical teaching. After that, foreign professors were brought in at different times [V. Duceschi (Córdoba), G. Nicolai (Córdoba), G. Viale (Rosario)] who gave courses in physiology, did research, and trained a few young men. Since these usually did not persevere, these professors exerted no permanent influence. Professor H. Piñero, who occupied the Chair of Physiology in the Buenos Aires University Medical School, was a brilliant orator and a stimulating teacher; he used to illustrate his lectures with experimental demonstrations, and made his students and assistants verify experimental work done in other countries, but he himself did no laboratory work. I was first a student demonstrator and then an instructor under him,

and performed my first experiments in his laboratory (1908–1910). When I was a student I used to make notes on each subject I studied as to what points I thought could be the subject of research. In 1908 I decided to work on the hypophysis and performed hypophysectomies on frogs and dogs, and observed the effects of posterior lobe extracts. In 1910 the Chair of Physiology at the School of Veterinary Medicine became vacant, and I was asked to give the course on a temporary appointment. I was then 23 years old and still in the last year of my medical studies. In 1912 I was appointed on my merits full professor. There I was able to do much experimental work with my students and collaborators in many species, even in cattle, horses, and goats, which were then easily obtained and kept at low cost.

University salaries were too small to provide a living, and there was no idea of the need for full time professors, even in the basic sciences. I worked part time in the Hospital, and from 1913 to 1917 I was head of one of the wards of Internal Medicine at the Alvear Hospital. Professor Rudolf Kraus, Director of the Public Health Laboratories, asked me, at that time, to direct the Department of Pathological Physiology and Endocrinology, which I did from 1915 to 1919. There I studied the action of snake, spider and scorpion venoms and the serum treatment of these poisons, and also investigated several problems of endocrinology.

I soon decided to concentrate my activities in one direction, and, though I was attracted by internal medicine, I chose physiology because I was able to do research in this subject, and I was convinced that I could thus be more useful to my country. In 1917 I discontinued my medical practice and in 1919, when appointed to the Chair of Physiology at the Medical School, I worked full time in teaching and research exclusively in my Department at the Medical School. Of course my income was reduced, but this was compensated by the great advantage to my work, and the deep intellectual satisfaction it brought. Thus I became the first full-time professor in the Medical School, and the example was followed by other medical schools in Argentina and several South American countries. My pupils Professors Lewis, Orías, Hug, Braun-Menéndez, Foglia, and others have also been full-time professors.

At one time the only activity considered proper for a medical graduate was that of a clinical practitioner or a surgeon. A laboratory had no other purpose but to carry out a few tests in aid of clinical diagnosis, tests which were not always considered as indispensable. No original research was done. The laboratory man was considered second-rate in respect of professional and social status, and his remuneration was correspondingly lower than that of his clinical collegaues. Medical schools were in the hands of men with large and time-absorbing practices, who had no knowledge of scientific methods or of the significance of research. The most powerful was frequently the President's personal physician, or the physician who had the largest number of members of Congress for patients, or might be himself a member of Congress.

A disastrous policy was that of beginning clinical instruction before the student had acquired basic medical knowledge. Empiric methods were over-

rated, and scientific method and reasoning were despised. Practical teaching and laboratory work in the basic sciences was not organized or was deficient. This system has long been prevalent not only in South America, but also in some of the European countries. This policy has been corrected in part after a long sustained struggle.

This exclusive preoccupation of medical schools with routine clinical instruction has been a principal cause of the poverty of original work in medical research in Latin America. Clinical professors gave most of their time and thought to their private patients and their problems. They were competent in the current diagnostic methods and treatment, but had no time for scientific training and research. They despised studies made on animals or isolated organs and tissues, and any results that had no immediate clinical application. They considered that the only physiological knowledge which was of importance, and which should be taught, was that which they knew or in which they were interested. They believed that original research was beyond the possibilities of their medical school and country. Richet's dictum that anyone who opposes physiology and clinical medicine understood neither one nor the other could be well applied to them.

In the last thirty years there has been considerable improvement. Many of the clinical professors apply the modern methods of physiology, biochemistry, and biophysics in diagnosis and treatment, and a few perform original research. Most of them now understand and respect experimental physiology and support its development.

The creation of the Institute of Physiology in the University Medical School of Buenos Aires in 1920, was of widespread importance; it became a center for teaching and research which irradiated its influence on medicine throughout Latin America. Theoretical and practical teaching of physiology, and biochemistry, and biophysics for the Schools of Medicine, Dentistry, and Pharmacy was centered in the Institute, in which seven major and several minor courses were given and there was a daily attendance of 200 students in the six teaching laboratories. The personnel, mostly voluntary, numbered 120, 35 of whom were doing original research. Some men from North and South America and from Europe came on fellowships to be trained or work there. It was thus possible to integrate and coordinate teaching and research in all these subjects with considerable economy and efficiency because duplication, repetition, and contradictions were avoided. A close technical and intellectual cooperation was obtained which was fruitful in many ways.

Besides the teaching given to the students in the different courses, more advanced ones were trained as student demonstrators. Many stayed later and worked from one to three years on a thesis for the doctorate, some of which were of considerable value. Many clinicians were trained in modern physiological and biochemical methods of research. This caused rapid progress in many aspects of clinical medicine, such as cardiology, endocrinolgoy, gastroenterology, metabolism, nutrition, etc.

From the sixteenth to the end of the nineteenth century medical knowl-

edge reached South America exclusively from European sources. European and South American physiologists established relations towards the end of the last century. Since 1920 these relations have been closer, and there is a two-way interchange of knowledge. The affiliation of the South American Societies of Biology to the Société de Biologie of Paris in 1920 was instrumental in giving an international hearing to South American experimental papers. Several European professors have given courses in physiology and have done research in South American countries. A few men were trained by them, but most of these did not continue to work in physiology. Modern physiological research in South America has been initially carried out mainly by native physiologists who had not been trained by European professors. These include Alvaro and Miguel Ozorio de Almeida in Brasil, B. A. Houssay in Argentina, and C. Cruz Coke and F. Hoffmann in Chile, who have worked exclusively in teaching and research and have trained many pupils. In addition, two European professors, A. Lipschütz and J. P. Suñer, have been active in Chile.

Argentine physiologists have established relations with those of the United States since the Rockefeller Foundation granted fellowships in 1926 and, later, funds for research. In Sao Paulo the Foundation supplied funds for the establishment of a model Medical School and an Institute of Hygiene with full-time professors who did research. The work done by the Rockefeller Foundation has been outstanding in past and present times. Later the Guggenheim Foundation awarded fellowships with excellent results, and more recently the Commonwealth Fund and the Kellogg Foundation have also awarded fellowships. Several professors in the United States have had a deep influence on South American physiology; first amongst these was Walter B. Cannon and then Carl J. Wiggers. Most of the full-time professors and research workers in physiology in South American have been partly trained by these men.

The majority of Latin American countries now have a certain number of competent physiologists active in teaching and research. Their number is increasing and the quality of their work is continually improving.

Trends in physiological teaching and research, and the future development of physiology are subjects of much discussion at the present time. It will be profitable to consider some of the points under discussion.

The teaching of physiology to medical students should not be limited to applied medical physiology. Undoubtedly, preference should be given to human physiology, but the functions of the human body cannot be well understood without some knowledge of general and comparative physiology. Functions in one species are known better when they have also been studied in other species, and at different levels of integration, i.e., in the cell, tissues, and organs, and in the whole organism. A large amount of knowledge of human physiology has been derived and is still belng obtained by the study of isolated cells, yeast, molds, and lower animals. It is a mistaken and harmful idea that, in a physiological laboratory attached to a medical school, only man and mammals should be the subject of study. The human

subject should be used as much as possible in class and laboratory teaching, but research on cells and lower animals should not be neglected. Physiology advances more rapidly in a country where there are teaching and research institutes not only in medical schools but also in Science and other University Schools or government and private research institutes for the study of animal and plant physiology, general physiology, biology, etc.

Physiology as a science is independent of medicine and, as such, should be studied in autonomous departments within the university without restricting it to its applications to medicine, animal husbandry, or agriculture. Medical school departments of physiology should not only investigate problems in human physiology or their immediate applications in medicine, even when in teaching the emphasis should be on these aspects. Human medicine has been and still is a most powerful stimulus for the study of physiology, and the departments in medical schools are the most numerous and best equipped and in some places the only centers of physiological research and teaching.

Physiology is that part of biological science which studies the functions of living organisms and the laws governing these functions. Whenever in the course of scientific or applied research the functions of a cell or a living organism are studied, consciously or unwittingly physiological research is being done. Physiological thought has profitably invaded all of the biological sciences: anatomy, histology, bacteriology, etc. This "cross fertilization" of scientific thought is the source of much new and important knowledge and has greatly enriched physiology.

Physiology is the main foundation of modern medicine, because knowledge of normal function is a necessary basis for the understanding and control of functional disturbances caused by disease. Up to the end of last century, and even during the first years of this century, medicine was based almost exclusively on anatomy. Anatomical dissection was the only fundamental training given to future physicians and surgeons; post mortem pathological anatomy guided clinical diagnosis, which was mainly anatomical. Even today all hospitals have a pathologist for the study of biopsies and post mortem pathological anatomy; only few have a physiological laboratory. The more advanced surgical and clinical departments have a physiological laboratory, which, however, does not always have a competent director.

At first research was not understood and no funds were provided for it; later it became "distinguished," and many clinical professors believed it was easy to do research work with the aid of poorly-trained and badly-paid assistants. As they had no clear idea of what scientific research is, and they had no knowledge of the scientific method, important original results were never obtained. It is bad not to do any research, but badly done research is even worse. A good school is difficult to create, bad schools arise easily and unfortunately they tend to be hardy and prolific. It is better to prevent their birth because once they have developed it is difficult to suppress them or to make them correct their evil ways.

Scientific research can be carried out anywhere if proper methods are

used. Important discoveries, some of which have had revolutionary effects on medicine, have been made by scientists who were not medical doctors. for example Pasteur and Roentgen, or by medical men who were exclusively researchers; others again have been made by medical practitioners. The important factor is not the diploma of the research worker or the place where he is working but the method he uses and the quality of his work.

The advancement of physiology and the introduction of new methods has caused first the development and then the segregation of many branches of physiology such as biochemistry, biophysics, nutrition, pharmacology, pathological physiology, immunology, neurophysiology, endocrinology, and the physiology of the circulation, of respiration, of exercise, etc. Within these branches there are an infinite number of special parts, e.g., hormones, vitamins, steroids, nerve physiology, etc. This development has led to the belief that physiology has become so divided into parts that soon it will no longer be a separate entity. In fact, physiology is passing through a crisis of growth as a result of the great development of some of its branches. This does not diminish, but increases the importance of physiology, and also it increases the difficulty of integrating the vast and ever greater amount of accumulated knowledge.

Physiology studies the phenomena of living organisms as revealed by changes in substance, structure, or energy; i.e., physical and chemical phenomena which can be studied by accurate scientific methods. All physiological phenomena have physical and chemical aspects, which should be the subject of research because they can be well understood and profitable results are obtained.

Living organisms have a characteristic set of properties, the study of which is the particular object of physiology. A living organism has a specific morphological, physical, and chemical structure; it builds up its own specific substance from varied nutritive materials and tenaciously holds constant this specificity of composition and structure. A living organism, therefore, forms itself. There is a continuous exchange of matter and energy between a living organism and its environment, which is also characteristic and specific for each organism. There are constant and balanced functional levels, which re-establish themselves when they are disturbed. Living organisms respond to internal and external stimuli in a way which is peculiar to each one of them. These responses tend to neutralize change, so as to restore the initial state of equilibrium, or they give rise to defensive phenomena. The organism's response to further applications of a stimulus is modified by its previous experience, this manifests itself by phenomena known as accommodation, habituation, immunity, anaphylaxis, memory or learning, conditioned reflexes, etc. Growth, development, reproduction, and heredity are also specific for each living organism.

The tendency to maintain a constant functional level is an outstanding feature of living organisms. Claude Bernard referred to this fact in a famous aphorism: "La fixité du milieu intérieur est la condition de la vie libre" (3). This idea was developed by Cannon (4) in the concept of homeostasis, which

he defined as "the coordinated physiological processes which maintain most of the steady states in the organism." This constancy is made possible by the regulation of each function and the balance maintained between the different functions. Many factors, some of which are antagonistic to each other, play a part in each one of the different functions, but regulating mechanisms maintain an equilibrium between them.

The organism responds as a whole, i.e., as a functional unit. This complex unit is regulated by humoral and nervous integrative mechanisms. The nervous system can receive stimuli from the environment or from within the body, but the organism responds as a unit. The organism can acquire new possibilities of response (immunity, conditioned reflexes, learning) or adaptation (high altitude, exercise, etc.).

Full understanding of a physiological phenomenon is obtained only by studying it from different points of view: (*a*) the fundamental functional phenomenon in elementary units (organs, tissues, cells); (*b*) the great functions such as circulation, respiration, metabolism, etc.; (*c*) regulation of functions and regulatory mechanisms; (*d*) correlation and integration of different functions, and final regulation and integration of all of them in the whole organism; (*e*) adaptation and acquisition of new powers of response.

Once the object of physiological studies has been clearly established it is easy to see there is no crisis in physiology and that it shows no signs of decay. There is, however, a rapid growth and differentiation into many branches, so that it is not possible for one person to have complete and detailed knowledge of the whole field. The integration of this huge amount of knowledge is becoming increasingly difficult, but it is ever more necessary to achieve this integration, and the achievement is by no means impossible. The teaching of physiology should comprise the five aspects outlined in the preceding paragraph.

The study of Physiology in its branches and techniques should always be preceded by a clear understanding of the object of this science and knowledge of the characteristics and general properties of living organisms. Biochemistry, for example, is attracting a large number of young workers because it is a relatively new science with efficient research instruments and techniques; it permits the simplification of complex problems and is fruitful in results; moreover, it has numerous practical applications, and there are many positions and opportunities available for those who wish to take up work in this science. Biochemistry is in part organic chemistry, and in part physiology; it studies molecular transformations in chemical systems, in tissue slices, in isolated surviving organs and in the whole organism, and the mechanisms which cause these transformations. Many of the most fruitful studies are made on tissue extracts, homogenates, brei, and in particles separated from cells; but after this analytical study of chemical processes has been completed it is necessary to investigate the part they play in the functioning of the organism, their integration with other functions, and their homeostasis. Thus we are back in physiology, which is continuously being advanced by studies of this nature.

Even before undertaking the study of the integration of biochemical processes in the whole organism, it is necessary to endeavor to understand the influence of cellular organization on the functioning of the numerous enzymatic and chemical systems (some of them apparently incompatible) which exist at the same time in the cellular cytoplasm and nucleus.

Gradually the study of function has progressed from larger to smaller structures: organs, tissues, cells, cellular organelles, particles, molecules, ions, electrons. Macroscopic methods have given place to microscopic, ultramicroscopic, and electronic microscopic methods. Research technique is becoming increasingly difficult, specialized and limited. Subdivision of science is necessary for progress, but this increases the importance of not forgetting that all this knowledge must finally be integrated in order to understand the functioning of the organism as a unit.

If the subdivisions of physiology became excessively numerous, it might be necessary to have "integrative physiologists." It would be much better, however, if all physiologists understood the basic general principles of physiology, before taking up specialized work.

There is no science without generalization. Scattered facts are as grains of dust or steel which can neither be counted nor held together; welded together these can be made into bricks and girders and put together in an orderly way so as to raise a building. Physiology requires more than the mere accumulation of isolated facts; these must be coordinated so as to understand the mechanism of their production. For example, the discovery of adrenergic and cholinergic chemical mediators has given a much clearer understanding of visceral innervation than could be obtained by an unending catalog of responses to sympathetic and parasympathetic stimulation.

Physiology sails its ship between the Charybdis of premature, undemonstrated generalization, and the Scylla of unimaginative fear of hypothesis and synthesis. In Latin America the greater danger is to be found in uncontrolled phantasy, and it is necessary to develop and train an acute critical mind, constantly asking: what is your evidence? which are your controls? In other, more advanced countries, there is an almost superstitious belief in facts and statistics and a horror of hypothesis. Imagination, one of the great powers of the mind, is not only desirable but absolutely necessary for the advancement of science. Hypotheses are valuable research instruments, their value increasing with their fertility in suggesting demonstrations. They are, however instruments which should rapidly become worn and outdated by being submitted to proof. Once they have been shown, by rigorous demonstration, to have been only a partly true or completely false interpretation of the facts, they should be discarded and replaced by new and fertile hypotheses. The imagination of the scientific worker should be constantly producing hypotheses, but his published papers should contain few.

Physiology is increasingly fruitful; the last half century has seen how it has brought to light the greater part of our knowledge of nutrition, endocrinology, neurophysiology, renal and cardiovascular physiology, vitamins,

hormones, etc. Thus to quote a few instances of advancement in the understanding of problems in which I have been interested: About 1915 to 1920 it was widely believed that endocrine glands had only an antitoxic function, and signs of endocrine insufficiency were attributed to toxic substances arising in the intestine or within the organism, which were not neutralized by the damaged or absent gland. Up to 1921 the only known function of the adrenals was the secretion of epinephrine; there was no knowledge of the functions of the adrenal cortex. The activity of posterior lobe hypophyseal extracts on blood pressure, the uterus, diuresis, and the chromatophores were known in 1921–28; but extracts of the anterior lobe of the hypophysis were considered to be completely inactive, because the effects were followed only during a few minutes or hours. The advancement of endocrinology requires the isolation and study of the activity of each and every hormone; it must be remembered, however, that in the organism these do not act in isolation, but under the influence of conditioning, permissive, or antagonistic factors. No function is dependent on a single hormone, even when a hormone has a dominant effect; functions are regulated not by one hormone alone but by a hormonal equilibrium, as has been demonstrated in carbohydrate metabolism and diabetes, in sexual function, in the development of the mammary gland and the secretion of milk, etc.

New methods and more and more complicated instruments have to be used in physiological work. Frequently a single worker cannot master all the techniques needed to solve a particular problem; the cooperation of experts in several fields becomes indispensable, and the development of the central idea guiding the work has to be carefully planned. Great original discoveries are, and always will be, the fruit of single outstanding minds; aid should be given to capable men rather than to elaborately-planned projects.

The advancement of science and of its applications requires the training of physiologists, capable of making new discoveries. A medical school will have no greatness, and it will not train physicians with a modern and progressive outlook if it has no competent professors of physiology. There is a close correlation between the scientific advancement of medicine in any country, and the degree of development of physiological studies it has reached and maintains.

The training of young men in physiology requires teachers, (i.e., trained physiologists), opportunities, and the possibility of a career in physiological work. A competent and stimulating teacher is a powerful, and often decisive, influence. He teaches mainly by the example of his vocation and enthusiasm, his moral and intellectual integrity, his devotion to science, his capacity to develop in his pupils imagination tempered by critical judgment, and the habit of hard and sustained work. A great teacher finds no pleasure equal to the sight of the awakening and maturing of young minds.

Once I visited two physiologists on the same day in the same building. One of them had a laboratory magnificently equipped, but he complained that there was no more interest in physiology among the young men and

that they did not come to work in his laboratory. The other had many students and he said that never before had young men shown such great enthusiasm for physiology. My personal experience is that a professor who works enthusiastically and takes an interest in young men and their work, will attract more pupils than he can deal with.

A sound basic general education is absolutely necessary for the future physiologist, who should also have a good knowledge of physics, chemistry, biology, histology, and mathematics. The training of a physiologist is strenuous and takes a long time, a fact that deters more than one hopeful candidate. In most countries positions open to physiologists are not numerous, and the higher ranks are reached at a relatively late age (5). Moreover, if a sufficiently important and well-paid post, such as a professorship, is not available, the physiologist of advancing years will find difficulty in taking up professional work for which he will need more special training. The physiologist's salary will usually be far below the remuneration earned by his class-mates who have taken up a practice or who are doing clinical teaching. In many countries physiologists are not given their due social prestige and consideration.

Many young men believe that physiological research is difficult and that all simple problems within the possibilities of a beginner have long been solved. There are on the contrary any number of opportunities for research. It is not advisable, however, to inquire into small details of problems which are being actively worked on by many groups; it is better to begin in an unexplored field, or to apply new and more penetrating methods to old problems, or to study a fundamental problem.

A vocation, deep and sincere, must give the initial impulse, and it must be sufficiently strong to assure perseverance in the face of unavoidable difficulties. Harsh circumstances, however, are not always a deterrent, but they have an unfavorable effect when they are such that only the hardiest can resist them, because even these would have gone further in more propitious conditions.

For many years physiology was pursued in South America under difficult conditions; but it is not a wise policy to expect men capable of resisting the most adverse circumstances miraculously to appear. Modern society cannot rely for its scientific advancement on the spontaneous generation of exceptional and almost heroic vocations. Of course, sometimes this does happen and the apparently impossible is achieved. Not infrequently a Professor is appointed but is not given sufficient staff, equipment, and supplies. Administrative duties, bureaucratic procedures, conferences, committees, reports, and visitors, which cannot always be avoided, eat up time and energy. In certain countries there is no security of tenure; scientists, however competent and outstanding they may be, are quite liable to be insultingly dismissed by ignorant dictators.

Funds for teaching and research have come from private and public sources. In the course of recent years they come increasingly from public

revenue. Universities and scientific institutions which receive these funds have been able to use them freely in certain countries (Great Britain, United States, etc.). The only reasonable control is that necessary to assure the money is spent for the general purpose for which it was given. In certain South American countries, where the state is usually the sole source of funds, economic pressure is sometimes exerted by politicians in order to influence the appointment of professors or of the university authorities, or to create new poor university schools for political reasons. The attempt to have research directed by incompetent bureaucratic organizations has not been unknown. South American universities should be given complete autonomy; they should be free from al. politicial pressure and certainly not used as ground for political propaganda and agitation.

It has been mentioned above that funds should be given to capable men rather than to paper projects; men should come first, then facilities for work, finally buildings. Unfortunately in Latin America there is a strong-rooted belief that scientists can be made off-hand, that it is only a question of money. The Rockefeller Foundation owes its success in Latin America to its policy of aiding competent men and not wasting its funds on official institutions tied down to routine.

Aid for scientific advancement in Latin America is of great importance (6). The immense spiritual and material wealth of the Continent has not yet been developed. The importance of this development for the welfare of the western hemisphere and of the whole world, should be understood by more developed countries. In a few years the population of Latin America will be greater than that of the United States. Geographical, historic, and economic factors have created a close interdependence between the American nations, and the need of a good neighbor policy. This interdependence will increase with time. Centers for the training of South American physiologists should be organized and put under the direction of teachers who have proved their worth. Young men who have shown promise should be awarded fellowships in order to be trained at these centers, and they should be assured suitable positions and means to carry on their scientific work when they return home. Foreign travel every five to seven years is a necessary stimulus and an excellent way to prevent mental stagnation. The purchase of instruments not manufactured locally, and subscriptions to scientific journals are serious problems in Latin American countries, because of the weakness of their currencies.

Personal and scientific relations between scientists of the different countries should be much closer than they are, and there should be greater reciprocal respect for their work. Funds should be available for the training centers mentioned above, for the meeting of symposia, and for subsidizing scientific journals that publish serious work done in South America. To further the development of Latin American countries would be to pursue a wise policy of enlightened self-interest by the more advanced countries.

A fundamental problem of modern science is the assurance of freedom

of research, discussion, and communication. Without this assurance free enterprise is restricted, orginality of thought decays, and truth is distorted. Science flourishes only in an environment of freedom. It must remain free of religious or political dogmas. Secrecy, loyalty oaths, personal discriminations of all natures, and compulsory planning are so many enemies of scientific advancement. It is regrettable that many countries in order to fight systems of oppression copy many of their methods and restrict human and academic freedom. This is no new development in the history of mankind; it was known in ancient Greece as it is seen today.

Space forbids the discussion of the importance of scientific communications by means of periodicals, books, symposia, congresses, etc., the difficulty of obtaining space for publication, and the excess of fragmentary publications. Differences in language create a serious problem, and there is a pernicious chauvinistic habit of referring only to papers published in one's own country, with deplorable disregard of papers published in other languages. The United States and Russia are great nations, but sometimes they seem to forget that their population is only 6 to 8 per cent of that of the world.

Science is a supranational activity, and its results should be used only for the benefit of mankind, not to harm it. The scientific fraternity should be bound by ever closer bonds, and should be an example of fraternity and peace for all mankind.

I am convinced that physiologists are proud to work at such a noble, vital, and rewarding field of activity, the future of which is immense and ever widening and attractive.

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# NEOPLASTIC GROWTH

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Studies on neoplastic growth have been reviewed in this series by Furth in 1944 (1). Since then the field has undergone a rapid expansion, which is also reflected by the increasing number of review articles covering different aspects of the subject. Comprehensive recent monographs and reviews are available dealing with the biochemistry of neoplastic tissue (2 to 11), carcinogenesis and the mode of action of carcinogenic compounds (9, 12 to 20), experimental leukemia research (21 to 26), the role of viruses in tumor induction (9, 27, 28), tumor progression and the development of autonomy (29 to 32), the immunogenetics of tumor transplantation (33 to 39), genetics and tumor formation (35, 39, 40), experimental studies on metastasis (41), the effects of chemicals, radiation, and viruses on tumors (9, 42 to 50), the effect of low temperatures on tumor cells (51), and the various types of available experimental tumors (9, 52, 53). A review of the entire field within the present space limits would at best amount to a condensed list of references. The author has therefore preferred to discuss some particular topics which do not seem to be fully covered by other recent surveys. Consideration has been given to studies on the growth characteristics of experimental tumors with particular reference to spontaneous or induced changes in these characteristics. No claim can be made for completeness and the selection of the subjects discussed has been determined to a large extent by the interest of the reviewer. The literature of 1950 to 1955 has been primarily considered, but earlier references are also included wherever necessary. The review of the literature was closed April 1, 1955.

## Measurement and Rate of Tumor-Cell Multiplication in Vivo

Previous indirect methods to follow the multiplication of neoplastic cells by measuring the weight, volume, projected area, or diameter of solid tumors (cf. 54) have recently been supplemented by more direct techniques which permit the actual enumeration of tumor cells when growing in the ascites form, i.e., in the form of free tumor cells or tumor-cell complexes, or both, in the peritoneal fluid. This approach has its limitations, since not all neoplasms can be converted to grow in this form, and in typical ascites tumors cell multiplication is not entirely restricted to the peritoneal fluid. At least in some ascites tumors, infiltration of solid tissues by tumor cells is late to appear and of limited extent. In such cases the total number of free tumor cells represents the great majority of all tumor cells in the animal, particularly during the earlier part of the growth cycle. This number can be determined with considerable accuracy. Such measurements were made by quantitative rinsing techniques, combined with quantitative and differential

cell counting (55, 56, 57), measurement of the dilution of an injected dye in combination with cell counting (58, 59, 60), measurement of the dilution of acriflavine-stained tumor cells by unstained cells (61), determination of deoxypentosenucleic acid content in combination with differential cell counting (62), and measurement of isotope dilution after the inoculation of $P^{32}$-labelled cells (63). The two latter methods are not limited in principle to ascites tumor cells and might be applicable to solid tumors, provided that certain basic conditions can be fulfilled. The deoxyribonucleic acid method requires a detailed knowledge of the distribution of deoxyribonucleic acid (DNA) per nucleus in the population and its possible variations during the growth cycle. The method of isotope dilution assumes that no isotope is lost from the cell during the interval between two successive divisions. If the isotope content of a particular cell fraction is measured, such as the nucleus or nuclear DNA, it has to be remembered that incorporation of activity may occur from other fractions after labelled cells have been inoculated into the animal and thereby may antagonize the geometric dilution of activity that results from cell division. Parallel measurements of cell multiplication by isotope dilution and some other independent method are needed to establish the reliability of the procedure.

The quantitative growth measurements performed on ascites tumors confirm the existence of considerable differences between the growth characteristics of various neoplasms, even if they are of similar morphogenetic type and grow in their own inbred strain of origin (60). The multiplication of Ehrlich and MCIM ascites tumor cells is characterized by continuously increasing generation time (55), while the ascitic variants of the Krebs 2 carcinoma (59), Rous sarcoma (57), and three mouse lymphomas (60) show exponential growth during the early part of their growth period, followed by gradual (Krebs 2) or more abrupt (lymphomas) decrease of the multiplication rate. Significant variations exist in the size of the maximum population, the tendency to infiltrate solid intra-abdominal tissues, the behavior of average cell volume and cell concentration during the growth cycle, and the occurrence of cell death and degeneration (60). Some ascites tumors grow progressively until they kill the mouse and, in spite of decreasing growth rate, the cells are able to maintain their original volume even during the terminal stages, and the proportion of dead or degenerating cells does not increase with time (55). Others respond to increasing population pressure by a decrease of cell volume and cell concentration, accompanied by increasing cell death (55, 60). This indicates that differences may exist between different tumor cells with regard to their ability to make use of available nutrients and to resist adverse conditions which result from crowding or accumulation of waste products.

The size of the inoculum has been shown to influence the growth of transplanted tumors in various ways. Regarding the smallest inoculum necessary for tumor transmission, it has been conclusively demonstrated that a variety of tumors are regularly transplantable with a single isolated

cell. Although this had already been shown for mouse leukemia by Furth & Kahn in 1937 (64), it was often believed that carcinomas and sarcomas cannot be transmitted with less than a certain minimal inoculum of considerable size (3). This belief was based on experiments performed with artificial tumor-cell suspensions and on heterozygous animals. It has been shown by MacDowell *et al.*, however, that small inocula may have an immunizing effect in the absence of genetic identity between tumor and host (65). Furthermore, mechanical disruption of solid tumors may inflict considerable damage upon the cells. In more recent work immunological reactions were minimized by using newborn mice for inoculation, and mechanical disruption was avoided by employing ascites tumors. Regular transmission could be obtained by means of single cells with the Yoshida rat ascites sarcoma (66) and the Ehrlich and Krebs 2 ascites carcinoma (37, 67, 68). A solid mammary carcinoma of the Strong A strain could also be transplanted with a single cell (69), demonstrating that the phenomenon is not limited to ascites tumors. Hauschka (67) has emphasized the great potentialities offered by single-cell clones for the analysis of tumor-cell populations.

Single-cell inoculations usually give rise to tumors in a small percentage of animals only. This may be attributable to random damage inflicted upon the cells during the isolation and injection procedure or to variations in their proliferative capacity or to both. The finding that cell types with a certain chromosome number have a greater probability to become established as single-cell clones than others (37) indicates that a genetically-determined variability may exist between tumor cells with regard to their ability for progressive growth and lends support to the "stem-cell" concept.

The relationship between the dose of inoculum and the percentage of takes was studied by Hewitt on mouse sarcomas (70). He found that the number of cells necessary to give rise to 50 per cent tumors is different for different neoplasms and probably varies with the closeness of the genetic relationship between tumor and host. When host and tumor were genetically different, there was also some variation depending on the site of inoculation; axillary tumors became larger than inguinal and grew in a larger percentage out of small inocula. Intramuscularly the percentage of takes was higher than it was subcutaneously. The latter difference disappeared when dealing with a tumor of an inbred strain. This is interpreted to mean that local resistance mechanisms are less-readily manifested in the intramuscular site than in the subcutaneous tissue. Hewitt also studied the effect of a large number of dead cells in the inoculum and found that it does not interfere with the detection of a small number of viable cells. Using mixtures of viable and x-ray-damaged Ehrlich ascites tumor cells, Révész (71) reported a different result. Depending on the proportions of viable and lethally-damaged cells, the proliferation of the former was stimulated or inhibited. A tenfold excess of killed cells stimulated growth, while an excess by a factor of about $2 \times 10^5$ inhibited the growth of the small viable inoculum completely. The latter occurrence was followed by resistance of the inoculated mice to-

wards further challenges with large viable inocula. As a result of genetic differences between tumor and host, the damaged cells have thus apparently succeeded in building up an effective immunity in the host sufficient to inhibit the growth of the small viable inoculum.

Although a critical inoculum level is thus apparently not a general requirement for tumor transmission, threshold phenomena may be of importance with regard to the form of growth. Certain ascites tumor cells have to be inoculated in large numbers to multiply in suspension form and will only grow in solid form out of small inocula (62, 72). When inoculum dose is varied within the range necessary for 100 per cent growth in ascites form, the latent period that elapses before cell multiplication starts is inversely related to the size of the inoculum (55). Analogous findings were made on microorganisms (73). There may also exist some relationship to the tissue-culture work of Earle and his associates who showed that very small inocula can grow only if the volume of the medium is restricted (74) and that very large inocula are required for growth in suspension (75). The critical inoculum level required for growth in the ascites form may decrease if the tumor is propagated in this form for more prolonged periods of time and may even reach single-cell level (72).

There seems to exist some relationship between the dose of inoculum and the rate of the subsequent proliferation. It has been demonstrated for tissue cultures (76), solid tumors (77), and ascites tumors (55) that optimum growth is obtained when medium-sized inocula are used. Major departures of the inoculum dose in either direction from the optimal results in a slight but consistent decrease of the multiplication rate.

Various factors were found capable of influencing the growth rate of certain established tumors in their hosts. Presence of the milk factor in the host was shown to enhance the growth of transplanted mammary adenocarcinomas of the C3H strain (78, 79). Another accelerant for a mouse mammary tumor was isolated from the tumor itself by Shear *et al.* (80, 81). The active factor was present in both the mitochondrial and the microsomal fraction and was not identical with the milk agent. It was ineffective unless injected from 6 to 31 days prior to tumor inoculation. This factor is probably related to the XYZ factor of Casey (82) and the enhancing activity of lyophilized tumor tissue studied by Snell, Kaliss, and others (38, 83).

The growth of a transplantable rat liver tumor and of the Walker 256 tumor was enhanced by liver regeneration in the same animal. There was no similar effect on the Murphy lymphosarcoma, and none of these tumors had any effect on liver regeneration (84). Liver regeneration in rats was also found to induce mitotic activity in the resting liver of a parabiotic partner, indicating the possibility for the transmission of a mitotic stimulus through the blood stream (85). In confirmation, injection of blood serum from hepatectomized animals was shown to induce mitotic activity in resting liver (86). The question arises as to the limits of the tissue specificity of this phenomenon and whether responsiveness of certain tumor types is in any

way correlated with their morphogenetic origin and their degree of differentiation. While nothing is known about the behavior of neoplastic cells in this respect, an impressive body of evidence indicates the existence of tissue-specific mitotic stimuli for normal cells. To quote a few representative cases, the work of Teir and his collaborators has shown that intraperitoneally injected suspensions of liver or orbital gland stimulates mitosis preferentially in the homologous organ of the rat (87). Tissue suspensions made of various portions of the alimentary canal exerted a mitosis-stimulating effect upon the epithelium of the stomach (88). Weiss (89) reported that the incorporation of organ fragments from 6-day-old chick embryos into the area vasculosa of 4-day-old chick embryo hosts results in a striking and relatively specific growth response by the homologous tissue of the host. He states that the available evidence supports the general principle of organ-specific chemical growth regulation by homologous cell constituents. Ebert (90) has confirmed these findings and has shown by isotope techniques that tissue-specific molecules larger than amino acids are being incorporated selectively from grafts into homologous host tissues. It would be of great interest to know whether or not neoplasms have retained the specific sensitivity of the tissue of origin to growth stimulation by homologous tissue extracts, or, alternatively, whether they have acquired a broader range of sensitivity including growth stimulation even by heterologous tissue constituents. Studies on tumors of similar origin but various degrees of anaplasia might be relevant in this respect.

The effect of growth hormone and adrenocortical hormones on experimental tumors has been reviewed in detail by Reid (91) and will not be surveyed here.

In contrast to the belief that mitotic rhythms are absent from malignant neoplasms, the careful studies of Voutilainen (92) demonstrated the existence of 24-hour rhythms in a number of human tumors. Maximum mitotic activity occurred usually during sleep. The rhythmic changes were essentially similar to those occurring in normal tissues.

## Host Range and Its Alterations

The genetic theory of tissue transplantation in general and tumor transplantation in particular has become firmly established (34, 36, 38, 39, 93). According to this theory, the ability of a transplanted tissue to maintain itself (and, in the special case of tumor tissue, to grow progressively) in the new host depends primarily on the simultaneous presence of certain genetic determinants, called histocompatibility (H) factors, in the host and in the transplant. A number of H-factors seems to exist in the mouse located on different chromosomes, and each locus can be occupied by any one member of an allelic series. A comparison of different mouse strains shows the existence of considerable genetic variation with respect to H-alleles, and, since transplants usually need the presence of compatible alleles at several H-loci in the host to maintain themselves, the chances are small that a tissue or a tumor will be transplantable to unrelated mice. In contrast, tumors of highly in-

bred homozygous mice are regularly transplantable to all members of the same strain (since they all contain the same histocompatibility factors as the donor animal) and also to $F_1$ hybrids between the strain of origin and any other resistant strain (since a single dose of each required H-allele is sufficient). The percentage of takes in $F_2$ or backcross hybrids depends on the number of histocompatibility factors required by the tumor, with the reservation that factors common to the original and the resistant strain will not be detectable. For a given cross, the number of H-factors that are different in the two strains and are required by the tumor can be determined from the percentage of progressive growth in segregating $F_2$ or resistant backcross hybrids, this percentage depending on the random Mendelian coincidence of segregating H-factors.

The knowledge of H-factors has advanced considerably in recent years, which is to a large extent due to the painstaking work of Snell and his colleagues (94 to 98). H-factors seem to operate by determining the specificity of isoantigens in fixed tissues and also in erythrocytes (34, 36). Recent evidence (99) indicates the existence of isoantigenic systems of considerable complexity, controlled by the H-factors, and reminiscent of the Rhesus system in man. When normal or neoplastic tissues are implanted into genetically unrelated hosts, their antigenicity provokes an immunological response that leads eventually to their destruction. Little is known about the exact nature of this response. The resistance factor has been shown to be passively transferable by lymphoid tissues, but not by blood, serum, or killed lymph nodes (100). Experiments with diffusion chambers of known pore size have demonstrated that the presence of host cells is necessary for the destruction of incompatible transplants *in vivo* (101). Host resistance can be broken down by whole-body Roentgen irradiation (102, 103), cortisone treatment (103, 104, 105), or pretreatment with lyophilized or otherwise killed tissues or tissue extracts, prepared from the tumor or from normal tissues of related genotypes (83, 106 to 110). The effects of x-rays and cortisone are probably attributable to immunological paralysis. The basically immunological nature of the "enhancing effect" of lyophilized tissue is indicated by the following findings (109): the injections have to be made before the period of active transplant growth; the effect persists and can be passively transferred; it is highly specific since enhancement occurs only if tumor and tissue used for pretreatment share a genetic factor that is lacking in the host; and the enhancing substance is probably a known isoantigen. The possible mechanism of the effect is discussed by Snell (109) who relates it to the findings of Billingham, Brent & Medawar concerning "actively acquired tolerance" to homotransplants (111).

While most normal tissues, leukemias, primary carcinomas and sarcomas, and transplanted tumors in their early transfers were found to behave in accordance with the genetic theory, a number of cases are known that appear to be exceptional at first sight. Serially transplanted tumors, particularly carcinomas and sarcomas and to a much smaller extent lym-

phomas and leukemias, often show a tendency to increase their initially limited host range and eventually acquire the capacity to grow in certain foreign genotypes and, in some cases, in all members of the species. At least the earlier phases of this change are correlated with a stepwise decrease of the H-factor requirement. Strong (112) has interpreted the changes as somatic mutations while Gorer (34) viewed them as an "antigenic simplification," with certain antigens crowding out other, weaker ones. A difference in the potency of different H-loci has been demonstrated (109).

The recent work of Hauschka and Levan, using cytologically favorable ascites tumors, revealed the existence of a correlation between host range and the distribution of chromosome number in the tumor-cell population (36, 37, 67, 113 to 116). Diploid tumors are usually very specific in their host requirements, while heteroploid ones can grow in a wider range of host genotypes. In certain cases the loss of strain specificity was paralleled by the development of heteroploidy in a tumor (37), and a small fraction of heteroploid cells could be selectively concentrated by propagation in a partially incompatible host (117, 118). No such effect could be observed when the immune response was weak as in newborn mice or when adult mice were inoculated with rapidly killing overwhelming inocula (117).

The question arises as to the nature of the change that is responsible for a simplification of the histocompatibility requirements. Hauschka views it as resulting from disturbances in genic balance consequent to the development of heteroploidy and leading to a decrease or loss of the antigenic end products which no longer elicit iso-antibody in protective titer. Loss of transplantation specificity would be the result of immunologic selection of the least antigenic types among the viable chromosomal variants of the graft (67). As alternative possibility, Hauschka & Schultz (116) discuss impaired sensitivity of heteroploid cells to antibodies. Both mechanisms may conceivably operate. Decreased isoantigenicity was demonstrated in a tetraploid line of the 6C3HED lymphosarcoma which, in contrast to the original diploid line, became able to grow across an H-2 allelic barrier in a foreign mouse strain (118). Studies of Snell *et al.* (97) show that even such old and apparently "non-specific" tumors as sarcomas 37 and 180 may have retained some isoantigenic specificity, since they grow more readily in mice containing a certain allele at the H-2 locus than in others. H-2 is a particularly "strong" locus, and a powerful deterrent is provided against progressive growth when host and tumor differ with regard to the H-2 allele they contain (97). The well-known fact that an effective immunity can be built up in mice of foreign genotypes against "nonspecific" tumors by pretreatment with small, damaged, or killed inocula also suggests that a certain degree of antigenicity is still retained by these neoplasms. According to Gorer, tumors growing in foreign strains are often doing so in spite of the presence of antibodies (119).

Antigenic differences between tumor and host would deserve greater consideration than usually given in experimental approaches to tumor therapy. Already Loeb (33) has pointed out that tumors growing in unrelated geno-

types often tend to regress after the application of an agent somewhat damaging to the tumor cells. This has been amply illustrated in the older literature. Some more recent studies have further elucidated the problem. Oughterson *et al.* (120, 121) demonstrated that a small dose of radiation which was rather ineffective against a mammary carcinoma grown in its own inbred strain, readily cured the tumor if growing in $F_1$ or $F_2$ hybrids produced by outcrossing the original strain with a resistant stock. Following up this work, Cohen & Cohen (79) demonstrated that the median lethal x-ray dose was variable for a mammary carcinoma of the milk-factor-harboring C3H strain, depending on the genotype of the host and the presence or absence of the milk factor. In pure C3H mice $LD_{50}$ was 5700 r. In C3H×CBA $F_1$ hybrids an $LD_{50}$ of 5100 and 3950 r, was found, respectively, depending on the presence (in the former case) or the absence (in the latter) of the milk factor. They have also shown that the radiosensitivity of the tumor decreases considerably when the resistance of the host is deranged by total-body irradiation (122). They suggest (79) that " . . . the radiosensitivity of a tumor is a quantitative measure of subliminal host-resistence resulting from immunogenetic differences in the host-tumor relationship, including extrachromosomal factors." The way in which regression may be brought about by an essentially nonlethal x-ray dose in a foreign host is suggested by the experiments of Révész (71), discussed above. A large proportion of damaged cells may immunize the host and thereby prevent the growth of a small proportion of remaining viable cells that would be perfectly capable of progressive growth in nonimmunized animals.

Changes in the host specificity of transplanted tumors are of great interest since they represent a characteristic case of "tumor progression" (30) and sometimes coincide with the changes in growth rate (123). It would be of considerable significance if the histocompatibility requirements of a tumor could be altered experimentally. Some promising work has been undertaken in this direction. The early work of Warner & Reinhard (124) indicates that small x-ray doses may increase the host range of transplanted mouse carcinomas. This finding might be related to the mutagenic effect of x-rays, and it is unfortunate that this interesting work has not been followed up. The recent experiments of Barrett & Deringer represent a different approach (125 to 129). These authors have shown that a transplanted mammary carcinoma of the C3H strain regularly undergoes a change leading to a higher percentage of takes in resistant backcross mice if kept for one or several transfer generations in $F_1$ hybrids, produced by outcrossing the C3H strain with another, resistant strain. The change occurs at an early stage during growth in the hybrid host, it does not increase with prolonged residence in the hybrid, and it is permanent even after the tumor has been returned to the C3H strain. It could be induced regularly and repeatedly in all of more than 20 trials. There was no general increase of transplantability to unrelated genotypes, and no change could be observed with regard to invasiveness or growth rate. A certain specificity could be ascribed to the

phenomenon: the enhancement of transplantability was greater when the backcross recipient and the $F_1$ hybrid donor corresponded with respect to the resistant strain which entered into the outcross, than when the resistant parent strain was different.

Using different tumors, Hauschka (67) confirmed the findings of Barrett & Deringer. He also observed some changes towards increased specificity (decreased percentage of takes in backcross mice) after residence in $F_1$ hybrids. This was a most unusual finding, since tumors usually proceed towards decreased specificity and changes in the reverse direction were not described previously (39). The most recent literature contains several analogous findings, however. The observations of Hauschka were confirmed by Klein *et al.* (130), using different tumor-host systems. After having carried a spindle-cell tumor in an alien strain for nine generations, Strong observed increased specificity (decreased percentage of takes in $F_2$ hybrids) upon returning the tumor into its original strain (131). An analogous observation was reported by Hauschka (37) who carried the 6C3HED lymphoma of the C3H strain through 69 serial transfers in the foreign Swiss stock. The tumor regresses in Swiss mice unless transplanted at an early stage after inoculation. When the Swiss subline was reinoculated into C3H mice after from 18 to 23 transfers, it appeared that survival time was significantly prolonged. After from 64 to 67 passages in Swiss, the tumor was inoculated into resistant backcross hybrids and showed a considerably decreased percentage of progressive growth.

Several questions arise in connection with these transformative mechanisms. Are the changes real adaptations, induced by the hybrid environment in the majority of the cell population? Alternatively, are we dealing with selection phenomena where certain pre-existing variant cells are particularly favored and selectively concentrated during passage through the hybrid? Even combinations of these two main mechanisms would be conceivable: selection of adaptable variants could be followed by a superimposed adaptation. Another, less probable, possibility consists of the induction of specific mutations by the environment in a small fraction of the population, followed by their subsequent selection.

There is no experimental evidence available to decide between these alternatives, and present interpretations must remain speculative. Considering the cases of decreased specificity after $F_1$ passage, selective mechanisms may be operative as suggested by Hauschka (67), based on preferential survival of less antigenic types in the hybrid environment. Alternatively, the change may have been induced by some particularities of this tumor-host system in a certain proportion of the tumor cells. Some interesting parallels are provided by the experiments of other authors. Under ordinary conditions the 6C3HED lymphoma cannot grow progressively in Swiss mice. Koprowski (132) found that the tumor may acquire the capacity to do so if passed through embryonic Swiss mice for one transfer generation. Stewart (133) has described the adaptation of Ak leukemia to unrelated strains after

passage through newborn animals of the foreign genotype. Krebs, Thordarson & Harbo (102) reported the adaptation of a mouse lymphosarcoma to foreign hosts after passage through mice that received total body x-irradiation to prevent regression. In all these cases, a tumor was exposed to a foreign genotype, but in an animal where immunological mechanisms were not fully functioning and could not bring about tumor regression. After such passage, it acquired the capacity to grow even in immunologically active adults. In the case of Barrett, Deringer & Hansen (125 to 129), the $F_1$ hybrid host cannot develop any efficient defense reaction against the C3H tumor, apparently because the histocompatibility factors of the latter are identical with those contained in the cells of the host itself in a single dose. On the other hand, the host contains some H-factors foreign to the tumor, and, as Medawar pointed out (134), the tumor is at least genetically qualified to react against its host. After having been exposed to the foreign H-factors in the heterozygous $F_1$ mouse, the tumor becomes able to grow in the presence of some of them even when homozygous, as indicated by the increased percentage of takes in $F_2$ and backcross hosts. Is it possible that we are dealing with the reverse of the "acquired tolerance" phenomenon (100, 109, 134) and that tumors may become "tolerant" towards the products of foreign H-factors if exposed to them under conditions where the immunological system of the host cannot bring about tumor regression, either because it is nonfunctional in general as in the embryo or in the irradiated mouse or because it shares the isoantigens of the tumor as in the $F_1$ hybrid?

Considering the cases of increased specificity, it is difficult to see how any selection pressure in the hybrid host could favor cells with increased histocompatibility requirements to such a degree that the original cells of lower specificity are brought to disappearance from the population in one transfer generation. A selection theory would have to postulate their disappearance, however, since remaining cells of the original type would be selectively concentrated again when the tumor is tested in $F_2$ or backcross hybrids and the change towards increased specificity could never be detected at the population level. It would appear more probable that the cells suffer some chronic damage, nongenetic in nature, during transfer in some hybrid genotypes, making them more sensitive to immunological reactions and resulting in a decreased percentage of takes when tested in segregating hybrids. Hoecker *et al.* (135) have actually demonstrated the transitory nature of similar modifications, produced in a number of leukemic lines by passage through unrelated hosts. The development of decreased specificity ("tolerance") or increased specificity ("intolerance") in a tumor after residence in foreign genotypes might perhaps depend on the intensity of the reaction between immunologically active products of tumor and host cells, in analogy with the reverse case of pretreatment of foreign hosts with lyophilized tissue where small doses produce immunity while large doses result in tolerance (95).

Induced changes of host specificity have been discussed and speculated

upon at some length because they represent one of the first attempts to modify tumor behavior at will. Much remains to be learned about the mechanism of the modifications that were described until present. Application of modern techniques of population analysis, such as isolation of single-cell clones (67), application of modified fluctation tests (62, 136), and chromosome analysis (114, 115, 137, 138) might prove useful in future studies.

### Transformation of Solid into Ascites Tumors

Studies by Hesse (139), Koch (140), Löwenthal & Jahn (141), Yoshida (142, 143), Goldie & Felix (144), Klein & Klein (62, 69, 72, 145 to 148), Graigie (149, 150), Makino *et al.* (151), Tanaka & Kano (152), Takeda *et al.* (153), Isaka (154), Bather (155), Shelton (156), and Fekete & Griffen (157) have shown that a number of animal tumors can grow in the form of suspended and dissociated free cells or small cell complexes in the peritoneal fluid, eliciting the production of ascites and reaching a high absolute and relative concentration within the fluid. The term "ascites tumor" has been used by most workers to describe this phenomenon. This term does not always denote a particular tumor type, but rather a form of growth in which a number of different tumors—although far from all—are able to grow under certain conditions. In the following, it will be referred to as the ascites form or A-form of tumor growth. The phenomenon of free cell growth is not limited to the peritoneal fluid, as shown by Goldie *et al.* (158), but can be best studied there (see 55 to 60).

A systematic study on a representative variety of 42 mouse tumors (147) led to their classification as (*a*) immediately convertible, (*b*) gradually convertible, and (*c*) inconvertible to A-form.

(*a*). The immediately convertible group comprises all lymphomas and leukemias so far tested and some highly anaplastic and rapidly growing carcinomas and sarcomas with long transplantation histories. The sudden development of the capacity for immediate conversion was observed in a mammary carcinoma of the inbred Strong A strain which was inconvertible during its early transplantation history (69).

(*b*). The case of gradual conversion is particularly interesting. It is a reproducible quality of certain tumor lines that occurs regularly under standardizable conditions and is usually extended through a sufficiently prolonged period of time. For these reasons it is presently one of the few cases of "progression" amenable to direct experimental study. Furthermore, comparisons between the original and the converted sublines of the same tumor, grown under similar environmental conditions, permit investigations on the morphological, biochemical, and physiological correlates of the change.

The gradually convertible group of tumors was found to consist of anaplastic carcinomas and sarcomas that were unable to grow in the A-form unless a certain number of serial intraperitoneal transfers were made with exudate fluid, containing desquamated tumor cells (145, 146). Some repre-

sentative tumors of this group were subjected to detailed analysis (62, 72, 147, 148, 159, 160). Results were compatible with the interpretation that the conversion process is essentially the selective concentration of a small number of variant cells, pre-existent in the original solid tumor, and possessing a discontinuously high survival value under the environmental conditions prevailing in the peritoneal fluid. The variant type is stable when grown as a pure population in the solid form after subcutaneous inoculation of ascites, but is subject to a slight negative selection when grown subcutaneously together with the original solid type. Reconstruction experiments with artificial mixtures led to a tentative estimate of the proportion of variant cells in the gradually convertible solid MC1M sarcoma, being of the probable order of $4\times10^{-7}$. For this tumor, the cellular change leading to the ability to grow in A-form was shown to be correlated with a reduced cellular adhesiveness, an increased tendency to metastasize to the lungs (62), an increased mobility in tissue culture (161), an improved capacity to survive in a starvation medium *in vitro*, and certain biochemical changes in the microsome fraction (72). As a working hypothesis, it was proposed that the variant cells represent mutants with less exacting nutritional requirements than the original type (160).

(*c*). The group of inconvertible tumors was found to include all highly differentiated and slowly growing neoplasms so far tested, but also some rapidly growing and anaplastic ones. In contrast to tumors of groups (*a*) and (*b*), cell suspensions prepared from tumors of category (*c*) could not maintain themselves within preestablished ascitic fluids *in vivo* (162).

Convertibility to A-form would thus appear to depend on a specific cellular change that may occur (but does not always occur) in rapidly growing and highly anaplastic carcinomas and sarcomas. In some tumors the frequency of the change is apparently high or it conveys selective advantage to the new cell type even under the original conditions of solid growth, thereby rendering the tumor immediately convertible. In others, the variants are kept at a low frequency by adverse selection and can be concentrated only by deliberately applied selective pressure during the process of gradual conversion. In still other tumors, the change does not occur at all, or it occurs at such low frequency that the changed cells either are lost during transplantation or they cannot be detected by present selective techniques. No definite relationship can be said to exist between type of convertibility and morphogenetic origin of the tumor, with the exception of lymphatic neoplasms which seem to be immediately convertible and do not require any specific cellular change to occur.

When ascites tumors are explanted and studied under various environmental conditions *in vitro* they may show characteristic changes in morphology and behavior (149, 150, 163, 164). These changes are promptly reversible and appear therefore to be of a modificative nature, induced by the environment.

### Development of Resistance against Radiation and Chemotherapeutics

Development of resistance is one of the major challenges in radiotherapy and tumor chemotherapy. Efforts have been made in recent years to analyze its mechanism. It could be demonstrated experimentally that the sensitivity of transplanted tumors decreases upon prolonged treatment with such agents as x-rays (165, 166), colchicine (167), analogues of dimethyldiaminobenzene (168), and triethylene melamine (169). The most informative studies in this field are represented by the extensive investigations of Law and his associates (170 to 180). Using transplanted mouse leukemias, originally inhibited by certain folic acid analogues and other antimetabolites, they have shown that prolonged treatment regularly results in the development of resistance and in some cases even dependence. Resistance towards a given antimetabolite may be accompanied by cross-resistance to another agent while the sensitivity towards still other drugs may remain unchanged or sometimes even increase. By modifying the fluctuation test of Luria & Delbrück (181) for leukemic cells, Law has shown (136) that resistance is not induced in sensitive cells by the specific agent. Resistant cell variants are pre-existent in the population at a low frequency, and they overgrow selectively the sensitive type during prolonged treatment. Increase in resistance occurs in a discrete, stepwise fashion, and the change is stable and irreversible even in the absence of the antagonist. These events are remarkably similar to the development of drug resistance in microorganisms.

Although the specific biochemical mechanisms responsible for the development of resistance are not known, several promising investigations have been made in this direction (26, 172, 173, 175, 177, 179, 182 to 188). It is probable that different mechanisms are operating in different cases. Summarizing available evidence, Law mentions the following possibilities (26): development of enzymic detoxications, elimination of an activating enzyme, increase in biosynthesis and concentration of the metabolite of the inhibited enzyme, change in cell permeability to an inhibitor, and development of alternative pathways of synthesis of the inhibited product or products. As a clear rationale for future therapeutic trials, he recommends the use of two or more independently acting antileukemic agents. The probability of selecting doubly resistant mutants is very small.

Besides hereditary variability, environmental factors may be of significance in determining the sensitivity of tumor cells towards therapeutic measures. Such factors as e.g. oxygen tension (189, 190), metabolic activity (191), and form of growth (164) have been shown to be of importance with regard to radiosensitivity. The presence of specific metabolites may modify cellular sensitivity towards administered antimetabolites. The distinction between genetic and environmental factors determining resistance towards treatment would appear to be of particular importance for the therapy of human tumors.

## Other Changes in the Characteristics of Established Tumors

The intricate problem of progression of host-dependent tumors to autonomy has been discussed recently by Furth (29, 192) and by Foulds (30). Regarding the development of autonomy in thyrotropic pituitary tumors, Furth suggests (192) that the autonomous variants may arise from dependent cells by a transformation similar to somatic mutation. This is indicated by the fact that first generation grafts of more than 13 primary thyrotropic tumors (induced by radiothyroidectomy) grew only in thyroidectomized hosts and never took in normal mice. On later transfers, the tumors usually became autonomous, i.e. they acquired the ability to grow in normal animals. Since normal, nonthyroidectomized mice would select out autonomous and suppress dependent cells, autonomous cells were probably not present in the first transfer and developed later by mutation. Furth considers the possibility that the accelerated growth rate, increasing histologic and cytologic dedifferentiation, and diminishing hormone production occurring in the course of successive passages in both dependent and autonomous tumors is caused by similar modifications, but he stresses that assays of single-cell progeny would be required to prove this assumption.

The significance of heterotransplantability to the anterior chamber of the eye and its relationship to the autonomy of human tumors has been discussed by Greene (193). According to Greene, heterotransplantability differentiates phases of tumor development rather than varieties of tumors. Nontransplantable tumors would represent early developmental phases while transplantability would correspond to a later, more autonomous stage.

Recently Dumbell & Rous (194) have tried to approach experimentally the question whether sequential changes in established tumors are of the same nature as the primary change leading from the normal to the neoplastic cell. They administered methylcholanthrene to mice bearing pulmonary adenomas and mammary adenocarcinomas. Since the growth and morphology of the tumors remained unaltered in spite of prolonged treatment, the authors suggest that sequential neoplastic changes differ in nature from primary ones. This would be a conclusion of considerable significance and more extensive experiments would be desirable on this point.

Interesting problems are posed by differences in the ability of different tumors to disseminate in the body. The frequency of metastasis depends on a number of extraneous factors, such as e.g. the site of the primary tumor (195, 196), its size and age (41, 197), some particularity of the host-tumor relationship that can be influenced by cortisone (198 to 201), etc., and the result of any particular experiment depends to a large extent on the method of study and the organ and tissue sites investigated. If allowance is made for these factors and the experimental conditions are standardized, it still remains true that tumors of closely similar origin and morphology may widely differ in their ability to disseminate in the body (41, 202, 203, 204), and it has been stated that the incidence of metastases depends more on the nature of the tumor than on any other factor (204).

If there is an inherent difference between closely related tumors with regard to dissemination, it is conceivable that variability may occur within the cell population of one tumor in this respect. By regularly transferring metastatic tumors, it should then be possible to select with regard to this characteristic and produce a tumor subline with a higher tendency to metastasize than the original neoplasm. Such an experiment was performed by Koch in 1939 (205) who transplanted lymph-node metastases of the Ehrlich carcinoma serially and found that the resulting subline showed an earlier and more regular spread to the lymph nodes than the original tumor. Working with two different solid sublines of the same C3H sarcoma, one always carried in the solid form and the other converted to ascites form and reimplanted subcutaneously, E. Klein recently found (62) these to differ markedly with regard to their ability to spread to the lungs. Evidence was presented to indicate that the more metastatic line was produced by selection from an originally heterogeneous tumor cell population.

In this connection it is of interest that local x-irradiation may increase the metastasizing ability of various experimental tumors (206, 207, 208). The possible mechanism of this effect is discussed by Essen & Kaplan (207), who consider the possibility of somatic mutation, but dismiss it because the metastatic tumors appearing after x-radiation did not show a higher metastasizing ability when transplanted than the original tumor. It may be pointed out, however, that one transfer generation may not be sufficient to concentrate any mutants with an increased metastasizing ability. The efficiency of selection will depend primarily on the frequency of the mutants, the selection pressure, and the frequency of back-mutations. Differences in metastasizing ability between different tumor cells are probably relative and not absolute, and selection pressure could hardly be expected to be so high as to achieve an absolute selection of a more metastatic cell type within one generation. Such new cell types may also have selective disadvantages at the original site and may therefore not show up in the experimental system of Essen & Kaplan. Experiments of Koch (205) and E. Klein (62) show that it is possible to produce tumor sublines with increased metastasizing ability if selection pressure is applied consistently and for a prolonged period of time.

Melanotic and amelanotic sublines have been obtained from the same original mouse melanoma by means of selective transfer (209). The transformation of transplanted carcinomas into sarcoma-like growth has been discussed elsewhere (9, 210, 211, 212); it is still not clear whether the carcinoma cells undergo "spindle-cell anaplasia" (212) or there is an induction of sarcoma by the carcinoma cells in their host. Use of genetic markers might contribute to the solution of this problem. *In vitro* transformations involving change of normal into malignant cells, loss of malignancy, change in antigenicity, and acquirement of the capacity to grow in agitated fluid medium were described by several authors (213 to 216). Particularly interesting are the studies of Sanford *et al.* (216) demonstrating the occurrence of two

different types of malignant transformation among the progeny of the same single isolated mouse fibroblast.

Cytological changes that may occur in transplanted tumors during prolonged propagation include increase in average cellular size, nucleolar volume and amount of chromatin (217), development of heteroploidy in initially diploid cell populations (37, 72), changes in the modal chromosome number of heteroploid tumors (137), and altered frequency of mitotic abnormalities (218). While most spontaneous mammary carcinomas of the mouse seem to have a predominantly diploid chromosome complement (219, 220), all transplanted mammary carcinomas with a long history turned out to be heteroploid when studied cytologically or cytochemically (37, 69, 72, 115, 146, 219 to 224). Two mammary carcinomas, both of the Strong A strain, were followed cytologically from an early stage in their history through a prolonged period of transplantation in the home strain. In both cases the initially diploid tumor changed to heteroploid (37, 72). Most other long-transplanted carcinomas and sarcomas are also characterized by heteroploid modal chromosome numbers. A plausible mechanism for the production of heteroploid cells is provided by the endomitotic phenomena described by Levan & Hauschka in several tumors (115). The fact that wholly diploid cell populations may be replaced regularly by heteroploid indicates that the latter possess some selective advantage under the conditions of serial passage. Probable reasons for this have been presented by Hauschka (37, 67). It is interesting that most lymphomas and leukemias do not behave in the same way: they usually remain purely diploid even after long periods of serial transfer (115, 146, 221, 224), although some exceptions have been noted (118, 156). This may be correlated with the finding of Levan & Hauschka (115) regarding the conspicuous absence of endomitoses from the two lymphomas they have studied.

Little is known about cytoplasmic changes during prolonged transplantation. According to Ludford (217), mitochondria tend to increase in number and become shorter and more granular. The Golgi system becomes larger. Since these changes occur simultaneously with an increase of nuclear volume, they may be secondary to the development of heteroploidy. Observations of Dmochowski (225) showed that a mammary tumor originally induced by the milk agent has lost the agent by its eighty-sixth transfer in agent-free mice.

This review has dealt primarily with permanent changes in the characteristics of tumor-cell populations. Besides these, there exist a number of cyclic, transient changes, correlated with the physiological age of the cells and affecting cell volume, nuclear morphology, glycogen content, etc. Such changes were studied by Hirono (226) and by Révész & Klein (60).

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# ENERGY METABOLISM

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## Energy Transfer in Biosynthesis

Fritz Lipmann (1) showed that, during the last two decades, metabolic research has developed from mainly "breakdown biochemistry" to emphasis on biosynthesis. He writes,

> In the living organism respiratory energy is transformed into potential chemical energy and distributed through carriers of active groupings. In the end result these carriers of active chemical fragments are condensing agents gluing together small building blocks to construct the chemical structures that are the medium of living organization.

One of these carriers, adenosinetriphosphate (ATP), is regarded by Kennedy (2) as a common energy transfer agent in all the various living cells. The energy transferred by this carrier has recently been measured by Podolsky & Kitzinger (3) who were using Benzinger's (4) microcalorimeter. The heat of formation of the pyrophosphate bond as such amounts to $4.80 \pm 0.10$ kcal./mole. This is the difference between the heat of hydrolysis of ATP by myosin of 16.4 kcal./mole and the heat of a corresponding neutralization without ATP hydrolysis of 11.6 kcal./mole.

Borsook & Deasy (5) observed that most of the energy required for protein synthesis is necessary for the formation of small peptides from amino acids.

In three Herter lectures, Krebs (6) showed that biosynthesis is not throughout just a reversal of catabolism. He demonstrated this thesis by the formation of glycogen from noncarbohydrate precursors as a particular example. He discussed the nature of ATP as a reducing and an oxidizing agent and its special advantage as "energy currency" available under various conditions: "as long as two interacting systems have suitable concentrations and redox potentials to yield sufficient free energy on interacting they can supply energy for phosphate bond synthesis." Krebs showed the steps of intermediary metabolism whereby chemical energy may be released for the formation of ATP.

Krebs' third lecture is entitled "An Excursion into the Borderland of Biochemistry and Philosophy." He feels that the wholesale dismissal of teleological consideration in biology is unjustified. He makes the assumption "that the arrangement of the energy producing mechanism is purposeful, for example that it is the primary purpose of the oxidative degradation of foodstuffs to generate phosphate bond energy."

I am rather unhappy about the term "energy producing" because it means producing that which by definition cannot be produced. Krebs' justi-

fication of teleology naturally stimulates a counter argument. Purpose requires a mind. I agree with Krebs that the best way, and perhaps even the only way, to understand a machine is to know its purpose. This is so because the engineer who constructed the machine has a mind similar to the mind of the observer who attempts to understand the machine.

But if this analogy is used in an attempt to explain some biological process teleologically, we presume that we understand the mind of a creator who designed and constructed organisms. Science then would be the effort to understand Nature in terms of the creator's purposes. It would be theology. Krebs disagrees with teleology in physics but accepts it in biology. This dualism, however, is unfortunate for science as well as for theology.

For science it would mean the postulate of a fundamental difference between living and nonliving material, a difference which, instead of becoming clearer with the advancement of enzymology and virus research, becomes more and more uncertain. For theology it would mean to limit God's purposes to biological phenomena, a limitation hardly acceptable to a theist.

Teleological concepts are deeply ingrained in our language. This is natural because our language is older than scientific thinking, and we might accept anthropomorphic terms such as these as poetical expressions and, if we want to be exact, use the term "as if" [Vaihinger (7)]. Even as consistent a conditionalist as Max Verworn (8) writes, ". . . dass die Organismen in zweckmässigster Weise an ihre Lebensverhältnisse angepasst sind. . . ." Zweckmässig really means "according to a purpose," so Verworn accepts the German "Zweck" but rejects the Greek "Telos." The German word is so familiar to him that he is not aware of its original meaning. He forgets, so to speak, the quotation mark or the restricting "as if"; he uses a poetic expression or slang. "Zweckmässig" to Verworn does not mean "purposeful" but "fitting conditions" so that certain processes (life) take place.

"Teleology is analogism, is pseudo explanation, it belongs in speculative philosophy but has no place in scientific philosophy," writes Hans Reichenbach (9). Krebs feels that teleology is a powerful tool for research. And if Krebs recommends a tool of research, we naturally are tempted to use it too, just as every less successful fisherman is eager to find out which bait the master who catches the most and the biggest fish is using. But, as Goethe says, "eines schickt sich nicht für alle"—one thing does not fit all alike. What for Krebs may be a help, for another scientist may be a hindrance.

Instead of asking "What purpose does ATP serve?" and answering, "Its purpose is to carry energy from the site of oxidation to a site of biosynthesis," one may ask "Is ATP a necessary condition for life?," and answer that biosynthesis, a necessary condition for life, requires energy and ATP is a carrier of chemical energy which makes a transfer of energy from catabolic to anabolic processes possible. If among the four billion spermatozoa of one ejaculate of a male some did not contain sufficient ATP, they could not reach the egg and we would never know of their existence. And if by chance a female were to produce eggs without ATP, there would be no offspring. The fact

that we can observe a living organism indicates that this organism fits the conditions which make life possible and, because we never see the billions that, for one reason or another, did not make the grade, for every one that does make it, the one we observe appears to us purposefully adapted.

Vitale, Nay & Hegsted (10) noted the loss of weight in undernourished rats and write

> Undoubtedly the loss of weight must be considered as one of the more effective adaptive mechanisms. The smaller body weight requires fewer calories for maintenance.

In teleological terms, the purpose of this adaptation, is presumably prolongation of the starving rat's life. With similar justification one might state that the loss of weight (involving the loss of surface area) of a water droplet subjected to evaporation must be considered as an adaptive mechanism, since it undoubtedly prolongs the existence of the droplet in comparison with what it would be if the original rate of evaporation remained constant.

Allen, Friedman & Weinhouse (11) write,

> It is logical to assume that organs having a multiplicity of substrates available from the arterial blood are equipped with the means of choosing for catabolism those which are most suitable for their needs.

The assumption is logical only insofar as it is teleological. Since choosing is an act of will, we would have to assume that these organs are equipped with will power. Obviously this passage is meant poetically and this "choosing" instead of being a teleological answer constitutes a biochemical problem.

Not the satisfaction but the dissatisfaction with this assumption stimulates biochemical research. If we observe what looks like an organ choosing substrates, we investigate the conditions and are not satisfied until we can describe the process in terms of chemical reactions rather than an act of will.

In a recent review on energy metabolism in this series, Spiegelman & Sussman (12) concentrated their discussion on biosynthesis at the cellular level. They write, "The only meaningful test of validity for mechanisms which propose to explain the properties and behavior of cells must come from properly designed experiments with intact viable and functioning cells." An animal physiologist may repeat this statement and just replace "cells" by "animals." And since the cellular level has been treated so adequately in that review, the rest of this review on energy metabolism will deal with the animal as a whole. I want to emphasize, however, that a new development in research on metabolic processes at the level of the mitochondria seems to me of great significance, and in the near future a review might be entitled energy metabolism at the mitochondrial level (13).

## Metabolic Rate

Considerable progress has been made in the measurement of factors which affect metabolic rate. Fat and water content can now be estimated in living animals from measurements of density (14) and by tracer dilution techniques.

The state of thyroid activity can be appraised from the measurement of the plasma protein-bound iodine.

Miller & Blyth (15) made use of these measurements in their study of the relation of lean body mass to metabolic rate of 48 college students with a range of body fat from 2.7 to 44 per cent and a range of lean body mass from 40 to 91 kg. All subjects had a normal thyroid activity. The observation of Miller & Blyth, that in a very fat individual the metabolic rate per unit lean body mass was the same as in a slim one, is somewhat in opposition to the earlier measurements at the Carnegie Nutrition Laboratory which led to Benedict's conclusion (16) that fat is not metabolically inert but an "energy demanding substance," by which expression he meant that addition of body fat on the average is accompanied by an increase in total metabolic rate.

Referring to the results of Miller & Blyth, Keys & Brozék (17) write,

> Popular belief in the superiority of the body surface as the unit of reference is scarcely justified. If allowance is made for the inert mass of the body, then the need for considering surface area or some similar complexity disappears.

If a mouse-physiologist or a rat- , man- , or cattle-physiologist is each interested only in the metabolic rate of his own special object of study, then each can express the metabolic rate as a linear function of body weight, or possibly, as an improvement, of lean body mass: $B = a + bW$, where $B$ = metabolic rate, $a + b$ = constants, and $W$ = weight (or lean body mass). However, physiologists are wise to take to heart the advice of Lewis (18),

> It is a dangerous thing to use any kind of mathematical equation unless we keep its meaning before us and are able to express this meaning without the symbolism which mathematics affords.

The linear equation above can be expressed in words as follows: The metabolic rate of a man is the sum of the metabolic rate, $a$ kcal. of a man without mass, and $b$ kcal. for each kg. of his body mass. If the physiologist is philosphically minded, he may be bothered by the meaning of the term $a$, the metabolic rate of a man without mass. He could rearrange the equation, remembering that the term $a$ has actually been calculated from the original data as $a = \overline{B} - b\overline{W}$, where $\overline{B}$ is the mean metabolic rate and $\overline{W}$ the mean body mass. Thus, $B = \overline{B} + b(W - \overline{W})$. The metabolic rate is the mean metabolic rate, $\overline{B}$, for a man with the mean body mass, $\overline{W}$, and $b$ kcal. for each kg. difference between the actual body mass of a man and the mean body mass per man of the group. This formulation makes sense and might satisfy each specialist in his own group.

But the curiosity of some physiologists goes beyond one species of animals. They are interested in comparative physiology. Suppose linear regression equations for metabolic rate and body mass were worked out for all animals. The biometrist would find that different equations were necessary for small and large dogs and that, on the other hand, the same equation could be used for small dogs, rabbits, and chickens, which have similar body mass. The biometrist would also note that the equation for large dogs would

fit pretty well the data for small sheep. Differences between the equations are thus much more related to differences in body size than to other differences such as those between mammal or bird, carnivore or ruminant. One might discover further that the ratio of the mean metabolic rate, $\overline{B}$, to body mass becomes smaller and smaller as body mass increases and so does the parameter $b$. One might even discover that on the average the relative change, $\Delta B/B$, of metabolic rate is proportional to the relative change, $\Delta W/W$, of body weight, or that the logarithm of metabolic rate is proportional to the logarithm of body weight. The question is, therefore, justified: Do these systematic changes indicate a general relation of the metabolic rate and the body mass of homeotherms? The answer is: Yes; there is, in fact, a surprisingly consistent relation which may be expressed by the simple approximation $B = 3W^{3/4}$. The hourly metabolic rate of fasting homeotherms, in kcal., is about 3 times the $\frac{3}{4}$ power of their body weight in kg. (19).

This relation for the range in weight from a 50 gm. mouse to a 500 kg. steer cannot be explained by differences in the content of lean body mass. The basal metabolic rate per unit weight of a 100 gm. rat is about five times as high as that of a 70 kg. man. If the metabolic rate per unit of lean body mass were constant and if the lean body mass of the man were, say, 80 per cent of his body weight, then the lean body mass of the rat would have to weigh four times as much as its whole body to produce the metabolic rate of the rat.

For men, with rather small differences in size, lean body mass could be predicted from metabolic rate, as was shown by Behnke (20) and confirmed by Miller & Blyth (21). But, for a comparison between man and rat, this calculation does not work because in this comparison the metabolic effect of change in body size is so much greater than that of change in body composition. Actually, rats have about the same range of fat percentage in their body as do men. Thus, the metabolic rate per unit lean body mass itself changes with the size of the animal. Lean body mass *per se* can therefore not determine metabolic rate, even though it undoubtedly will become an important criterion for differences in metabolic rate within a given body size. In this role, it might even replace specific stature (height/weight$^{1/3}$) and possibly age, as suggested by Keys & Brozék (17).

Within groups of animals with small differences of body size the statement of Keys & Brozék is correct, that there is no necessity to express metabolic rate in terms of a metabolic body size such as the $\frac{3}{4}$ power of weight (or of lean body mass). The point is, however, that there is an opportunity to do so. There is no necessity to assume that, within man or within rats, body size has a different effect on metabolic rate from what it has between man and rat. We are, therefore, free to choose the simpler assumption, and to postulate that the same influence of body size exists within as between species and that the best fitting unit of metabolic body size, namely the $\frac{3}{4}$ power of body weight, (possibly $\frac{3}{4}$ power of lean body mass instead) may be used intra- as well as inter-specifically. Certainly the one equation $B = kW^{3/4}$ for all homeo-

therms is simpler than a multitude of linear regression equations each with two empirical constants in which size effects and special group and age differences are mixed. For comparative physiology it is more meaningful to express the metabolic rate, even within one species, in such a way that it fits into the general rule. The parameter $k$, the metabolic rate per unit metabolic body size, for detailed work may still be different for different groups of animals. It is 71 for men and 66 for women of Benedict's groups (19). Modifying terms with definite physiological meaning, such as age, specific stature, and, possibly now, fat content, may easily be combined with this formula.

Lamb & Michie (22) measured the basal metabolism of 19 children from two to ten years old and concluded that the results were more constant when expressed per unit height than per unit of weight or surface area. They might have expressed the metabolic rate per $kg.^{3/4}$ and noted the effect of age on this index of metabolic rate.

Brendel, Koppermann & Thauer (23) investigated the effect of narcosis on the metabolic rate of dogs and concluded that in big animals narcosis does not lower the metabolic rate below the minimum rate observed without narcosis. They explain observations on specific lowering of metabolic rate by narcosis in small animals by the greater relative part which the work of respiration plays in the total basal metabolic rate (4 to 12 per cent in dogs against 88 per cent in rats!). The basis of Brendel's argument, namely that 88 per cent of the energy of rat metabolism is required for work of respiration, was reported by Bänder (24). It is a typical result of misunderstanding of the meaning of results from statistical calculations (see Lewis' remark, page 38). Bänder expressed the relation between respiratory frequency and oxygen consumption as a linear regression. He deduced that the extrapolated rate of $O_2$ consumption for a rat "without breathing" represented the energy spent for requirements other than breathing, and that the total increase of $O_2$ consumption with increased frequency of breathing indicated the energy spent for the work of breathing. If he had also counted the frequency of heart beat and calculated a linear regression of metabolic rate on this frequency, he might have found that, in addition to the 88 per cent of the $O_2$ consumption required for work of respiration, there is another need of possibly 90 per cent for the work of the heart!

Holaday & Israel (25), somewhat in contrast to the conclusion of Brendel *et al.* mentioned above, report a large increase in work requirement for breathing under narcosis. This increased work, according to them, results from changes in the elastic properties of lung and chest wall.

One of the few errors which Lavoisier made as a scientist was his contention that the oxidation of the fuel for animal heat occurred in the lung. Recent observations by Bostroem & Lochner (26) on the oxygen consumption of dogs' lungs *in situ* do not indicate that Lavoisier was completely right after all, but they show that the oxidation in the lung can indeed contribute a considerable part (10 to 30 per cent) to the total oxygen consumption of the animal.

The idea that the metabolic rate of tissues *in vitro* still reflects effects of the metabolic regulator in the intact animal (19) received confirmation by the observation of Denison *et al.* (27) that tissues from rats exposed to cold for 50 days had a 35 to 55 per cent higher metabolic rate *in vitro* than did tissues from rats not exposed to cold.

Enzyme concentration and metabolic rate of tissues are positively correlated. Kunkel & Campbell (28) observed that in mouse, rat, and dog liver, the smaller the animal, the higher the cytochrome oxidase concentration. Similarly, Fried & Tipton (29) noted that the maximum activities of succinoxidase and malic dehydrogenase in heart, kidney, liver, and brain tissue of cow, rat, and mouse reflected the inverse relationship of metabolic rate per unit weight, and body weight.

## Temperature Regulation

The mean body temperature is the result of a balance between rate of heat production and rate of heat loss. The rate of heat loss is under the control of "physical temperature regulation." In low environmental temperature the rate of heat production is adapted to the rate of heat loss by change in metabolic rate under the control of "chemical temperature regulation" (Rubner).

The insulating quality of the body covering is changed mainly by changing blood circulation under vasomotor control. Hensel & Bock (30) measured the heat conductivity of human muscles as a function of blood circulation and concluded that the heat conductivity of the muscle as such is not altered by changing circulation. The increase in the rate of heat flow through tissue with increase in circulation is therefore only a change in convection.

Hensel & Bock found that the heat conductivity of human blood is the same as that of human muscle without blood circulation, namely $1.14 \times 10^{-3}$ cal., $cm.^{-1}$, $sec.^{-1}$, deg. $C.^{-1}$. The major role in physical temperature regulation, aside from changes in the insulating power of the body cover, is played by changes in the rate of water evaporation.

Zöllner, Thauer & Kaufmann (31) measured the insensible perspiration, and particularly the loss of water vapor, of nude men. With constant vapor tension of the environmental air the rate of loss of water vapor increases with increasing skin temperature. This increase is especially rapid when the skin temperature rises above 35°C.

Thauer, Zöllner & Kaufmann (32) differentiated between the loss of water vapor from the skin and that from the respiratory system. Concerning the latter, they noted that, with a temperature range of the inspired air from 7° to 35°C., the expired air does not reach 100 per cent relative humidity (the relative humidity apparently is meant for air which would have rectal temperature. The dew point of expired air thus is below rectal temperature).

Kerslake (33) noted a discrepancy between observations and one hypothesis that the rate of sweating is controlled by body temperature, as well as the other, that it is controlled by the surface temperature of the skin. He

then developed the hypothesis that rate of sweating in man depends on the temperature of nerve endings situated at some depth inside the skin. He calculated this temperature from the surface temperature and the rate of heat flow through the skin.

Ladell (34) studied the effects of water and salt intake on men working in a hot and humid climate. He observed that, without water intake and equally without intake of sodium chloride, thermal equilibrium was established at a temperature level above that maintained if the men drank water and took in salt.

A similar change in the level of body temperature has been observed in camels by Schmidt-Nielsen *et al.* (35). They found that the rectal temperature of the camel during a day may rise from 34.2° to 40.7°C. They regard this change, not as a failure of temperature regulation, but as a well-functioning mechanism which conserves water. The daily variations of body temperature in camels with free access to water does not exceed 2°C.

The ability to endure a hot climate is important also for other domestic animals, especially cattle. This ability varies greatly and depends on genetic factors. Klemm & Robinson (36) studied the heat tolerance of two breeds of calves from 1 to 12 months of age. They compared the reactions of two Australian Shorthorn bulls with those of two Zebu Hereford cross bulls during exposure to environmental temperatures ranging from 86° to 108.5°F. and absolute humidities from 6.5 to 16.6 gr./cu. ft. dry air. (Presumably in grains per cubic foot, this would be 14.8 to 38.8 grams water vapor per cubic meter dry air.) With a constant absolute humidity of 12.5 gr./cu. ft. (28.6 gm. $H_2O$ per cu. m. dry air) the temperature was raised in five steps from 86°F. to 108.5°F. Constancy of absolute humidity with changing temperature, of course, means changing relative humidity. The rise in temperature in the trials mentioned presumably decreased the relative humidity from 91 per cent to 44 per cent. In 4 hr. of exposure to 108.5°F., the rectal temperature of the Zebu Hereford cross bulls began to rise slowly and reached 104°F. in 8 hr. Under the same conditions, the Australian Illawara Shorthorn bulls showed a rapidly increasing rectal temperature which amounted to 105°F. after 3 hr. of exposure, and to 106° after 5 hr. The superiority of the Zebu strain in enduring a hot climate is attributed to its greater ability to sweat and also to lower its metabolic rate. The latter is also listed as a contributing factor for greater heat tolerance of lactating Brahman cows in comparison with Jersey and particularly Holstein cows [Brody *et al.* (37)].

For homeotherms, a cold climate means a greater thermostatic heat requirement than does a warm climate. What counts, however, is the microclimate. This was demonstrated by the study of Rodahl (38), who observed that military personnel in Alaska consumed about the same amount of food in this cold region as they would have consumed in the temperate climate of the United States. The energy intake of the Eskimos was approximately on the same level. Housing and clothing apparently produce in Alaska a microclimate similar to that we have here in the United States so that for thermostasis no significant increase in heat production is required. Men change

their microclimate so that they do not have to shiver. The degree of this shivering, however, when it occurs, seems not to be an index for a definite metabolic rate. Burton, Leach & Snyder (39) exposed nude men to 48°F. with 80 per cent and with 30 per cent relative humidity. The metabolic rate was unaffected by the change in humidity, but the men shivered more violently when the humidity was low.

*Hibernation.*—One way of solving a difficult problem is to wait until the problem disappears. Some animals are able to apply this method to the problem of maintaining a constant body temperature in a cold environment; they give up the constant body temperature and go to sleep until times get better.

Kayser (40) has written an extensive review on hibernation, based on his observations and those of other workers. He notes that hibernation is far from just a breakdown of temperature control. It is a regulation at a minimum level with specific metabolic rules. A characteristic change occurs in the ratio of liver glycogen to blood glucose level in the opposite direction to the change observed in diabetes. Lack of water contributes to an early onset of hibernation. Thyroidectomy does not produce hibernation, but injection of thyroxin shortens the duration of sleep.

A difference between hibernating animals and true homeotherms is noted in the behavior of their nervous systems. The nerve of a true homeotherm does not conduct impulses below a temperature of 9°C. The nerve of a hibernant, however, conducts to a temperature as low as 3.5°C. The bioelectric activity of the brain and shivering in the homeotherm disappear at temperatures below 15°C., whereas in hibernating animals those reactions can be noted at a temperature as low as 6 to 7°C. The heart of a rat stops beating when the temperature sinks below 9°C., whereas the heart of a hibernant does not stop until the temperature is lowered to 2° or 3°C.

The metabolic rate of hibernating animals weighing from 7 gm. (pipistrelle) to 2.1 kg. (marmot) appears to be almost proportional to body weight, namely about 2 kcal. per kg. body weight per day. Thus, the smaller the hibernating animal, the greater the decrease of ordinary metabolic rate.

Kayser notes that hibernating animals, in contrast to artificially cooled nonhibernating homeotherms, have a chemical temperature regulation even during hibernation. A European ground squirrel (spermaphile) consumed 20 ml. $O_2$ per kg. body weight per hr. at 5°C., and 39 ml. at 2°C.

## Regulation of Food Intake

According to Adolph (41) rate of food intake is one of the best regulated animal functions for quasi-constant condition. The daily rate of food intake of small animals such as rats shows a coefficient of variation of only 10 per cent. This function offers many possibilities of measurement for a study of the nature of animal regulations. The practical importance of the regulation of food intake in medicine is obvious, since overeating is, and apparently becomes more and more, a major health problem. In agriculture, on the contrary, the problem is to breed animals that do overeat, if by overeating one

means eating more than is required for the maintenance and reproduction of animals. One expression for the success of animal production is the total energy efficiency of the animal (energy in gained animal product/ingested food energy).

This quotient may be expressed as the product of two factors: $G/U = \Delta G/\Delta U(1 - M/U)$, where $G$ stands for energy in gain, $U$ for food energy ingested, $\Delta G/\Delta U$ for partial efficiency (or the amount of net energy per unit food energy in the ration), and $M$ for maintenance requirement. Total efficiency is greater the larger the ratio $U/M$, that is the more the animal eats in terms of its maintenance requirement.

Animals periodically stop eating even when their food supply is unlimited. In the course of eating they lose their urge to eat. That urge must therefore depend on a condition which is changed by food intake. What is this condition and how is it changed to stop the urge to eat?

When Adolph (41) diluted the diet of rats with undigestible cellulose or with water the rats consumed more, so that their energy intake remained nearly at the same level provided that the dilution of the diet did not go below a nutrient concentration of 17 per cent. Adolph concluded "within limits rats eat for calories." Peterson, Grau & Peek (42) came to a similar conclusion with chicks. When glucose was replaced by cellu flour (with a low digestibility), the chicks ate more, apparently to satisfy their energy requirement.

Earlier, in 1936, Kleiber (43) attempted to clarify the interaction of the rates of energy utilization, heat production, and food intake in animals. As an analogue for the energy transfer in the animal body he designed a hydraulic scheme which showed how the concentration of metabolites in the bloodstream may depend on the rate of absorption and the rate of utilization and catabolism, and how lactation rate, growth rate, and rate of catabolism might affect this concentration of metabolites in the bloodstream and, through this concentration, regulate the food intake. The scheme also indicated how the cooling power, or, if this was insufficient, the rise in body temperature, might affect the regulator of food intake. This scheme was a, perhaps premature, attempt to integrate the two theories on regulation of food intake which today compete for emphasis: the theory of a thermosensitive, and that of a chemosensitive regulator.

*Thermostatic regulation of food intake.*—Brobeck wrote, "Animals eat to keep warm and stop eating to prevent hyperthermia." In technological rather than teleological language, that statement says that food intake is controlled by a thermosensitive regulator.

Strominger & Brobeck (44) measured the effect of adding fat to a diet on the food intake of rats. On the first day after the change the rats on the high fat diet consumed much more food energy than they had taken in before. The estimated calorigenic effect of the food consumed was, however, nearly the same for the low fat and the high fat regime. Based on this observation, Strominger & Brobeck concluded that the specific dynamic action of the ration, rather than its content of chemical energy, determined the amount of food eaten.

The first day's intake of a newly changed diet may not be representative of the normal regulation of food intake; that may depend on the degree of curiosity in the experimental animals. Ulvesli, Husby & Presthegge (45) report that goats took their rations much more readily if an opportunity was given to change the feed.

In later trials, Strominger, Brobeck & Cort (46) extended their observations of food intake to a period of three weeks. They noted a sharp drop in food intake on the high fat diet after the first day, but they still confirmed the earlier result that rats on a high fat diet consumed more energy than they did with the low fat basal diet.

Strominger *et al.* noted that the rats in their trials had a rather low protein intake. Even the basic rations contained considerably less protein than the rats of Warkentin, Warkentin & Ivy (47) had taken by free selection, namely a protein content of 28 to 32 per cent of the diet up to the age of four months and even 45 per cent when they were older. One might speculate that the rats on the high fat diet ate more, stimulated by a "hunger for protein."

The theory of Strominger *et al.* fits an observation on steers in the hot Imperial Valley of California. Ittner, Bond & Kelly (48) fed two groups of steers *ad libitum*. One group received a ration high in concentrates, the other a ration high in roughage. The steers on the high roughage ration took in less digestible energy per day than did those consuming the concentrated feed, and less than steers in a cooler climate would have consumed. From Ittner's feed records we can estimate, according to Armsby's calculation, that the feed consumed daily by the two groups of steers produced nearly the same heat increment.

It is unfortunate that the calorigenic effect, which obviously is the major category of interest in the theory of Strominger *et al.*, was not measured but estimated. Such an estimate is very uncertain, especially since, according to Rubner's compensation theory, the specific dynamic action changes as a function of the environmental temperature if the experiment is carried out below the critical temperature of the fasting animal, which means for rats below 30°C. [Black & Swift (49)]. Furthermore, the specific dynamic effect of a day's ration in rats kept below 30°C. would depend on the time of food intake. It would be higher if the rat ate the food in a short time than it would be if consumption were extended over a greater part of the day. A serious criticism against the general applicability of the theory that specific dynamic effect controls food intake is the observation that the addition of thyroprotein, or substances with similar effect, to a ration usually increases food intake. Since the addition of the drug at the same time increases the specific dynamic action of the ration, it should, according to Strominger, decrease food intake. Thyroxin-like drugs are not only calorigenic but also thermogenic (increasing body temperature); they increase also metabolic rate and food intake. This effect does not fit the theory that food intake is controlled by a thermosensitive regulator, but it fits the competing theory of a chemósensitive regulator of food intake.

According to Kleiber's scheme (43) mentioned above, a higher specific dynamic action may lead to reduced food intake when cooling power is

limited, but to increased food intake when the cooling power is not the limiting factor, because the increase in catabolism lowers the level of metabolites in the blood.

*Chemostatic regulations of food intake.*—Bulatao & Carlson (50) observed in dogs with gastric fistula that gastric hunger contractions were produced by hypoglycemia and prevented by hyperglycemia. Mayer (51) demonstrated that the glucose concentration in the blood is a major condition which determines the urge to eat. Increases in blood glucose levels, produced by injections of glucose, or of fructose or epinephrine, decreased food intake. Decreases in the levels of blood glucose following insulin injections increased food intake. Injections of sucrose or fat emulsions which did not affect the level of blood glucose also did not affect the rate of food intake. Hyperglycemia maintained by three daily injections of glucose into alloxan-treated hypophysectomized rats made these rats starve to death for lack of the urge to eat the food that was available to them.

Apparent exceptions to the theory that blood glucose level is the major determinant of food intake, such as hyperphagia in diabetes, led Mayer to the postulate that phosphorylation was necessary for blood glucose to cross the membranes of the hypothalamus cells which transmit the impulses from glucose levels to the centers that activate eating.

Because it was difficult to measure glucose levels in the hypothalamic center, Mayer substituted the arteriovenous difference in glucose concentration in man, $\Delta$ glucose, measured peripherally between the blood from a finger and that from the antecubital vein as an index for the "effective glucose concentration." This substitution rests on the assumption that the rate of glucose utilization in the organ, across which the $\Delta$ glucose is measured, is determined by, and is, therefore, a valid index of, the effective glucose concentration in the arterial blood. This seems a questionable assumption: local influences may change the rate of circulation or the metabolic rate of the peripheral organ and that may lead to a change in $\Delta$ glucose even with a constant effective glucose level (effective for the hypothalamus).

Mayer discusses such a change. Injection of epinephrine eliminates hunger but decreases the peripheral arteriovenous difference in glucose level. As Mayer points out, the carotid-jugular difference in glucose level in this case would have been a more consistent index for the effective glucose level in the regulation of food intake than the peripheral $\Delta$ glucose.

Mayer's theory of the glucostatic control appears today the best founded theory for the regulation of food intake. This theory gets support in general from the investigations of Kennedy (52) who writes "The experimental findings thus lend support to the concept of the control of food intake by a chemosensitive rather than a thermosensitive mechanism." Kennedy, however, does not agree with limiting the effective metabolite to glucose and writes,

> . . . wide variations in chemical composition of the diet are without effect on the calorie intake unless palatability is affected. This suggests that control of intake is

dependent on the whole complex of metabolites in the bloodstream rather than on glucose alone.

If protein hunger increases food intake, as one might speculate from Strominger's results, then the concentration of amino acids or similar protein precursors in the blood might affect food intake. Mayer maintains that glucose differs from fat and protein because the body stores of fat and protein are so large that a change from one feeding to the next would be an insignificant part of this store. This reasoning is undoubtedly sound, but we know now that a body store may not necessarily form one kinetic pool, and it is possible that only a small fraction of the body protein and the body fat form a mobile pool in the same way that about 1 per cent of the skeletal phosphorus of a cow is a "mobile P pool" (53).

Ruminants absorb practically no glucose, as most of the carbohydrates of their food are fermented in the rumen and the fermentation products, lower fatty acids, are absorbed into the bloodstream and are the major precursors for the blood glucose (54). The question arises, therefore, whether or not in ruminants the blood concentration of another metabolite, such as acetate, instead of glucose might be the regulator of food intake. High-producing dairy cows sometimes seem unable to produce enough glucose as precursor for the lactose in the milk and then suffer from ketosis. Those cows lose appetite which makes their condition worse. Injection of glucose (or propionate) usually restores their appetite. In this case, then, injection of glucose increases food intake, quite the opposite from the effect in normal nonruminants.

The hypothalamus seems to contain two opposing regulators of food intake. Thus, Anand, Dua & Schoenberg (55) recently reported that bilateral destruction of the lateral part of a region in the hypothalamus of cats and monkeys produced aphagia and that destruction of the medial part led to hyperphagia only when the lateral part was intact. According to Kennedy (56), the hyperphagia exhibited by rats with hypothalamic lesions represents a primitive unconditioned urge which may be described as hunger. The effect of this urge may, however, be modified by other influences, and Bruce & Kennedy (57) distinguished two types of urges to eat: (*a*) hunger, the central regulator of which is located in the hypothalamus and which is also called the calorimetric factor of urge for food intake; (*b*) appetite, the central regulator of which is situated in the cortex of the brain and the influence of which may modify, in some cases even reverse, the effect of the hypothalamic factor. Appetite has to do with "palatability" of the food.

Kennedy (52) notes that in young rats hunger seems to dominate food intake. Older rats become more discriminating, appetite becomes more important. This is particularly true for obese rats which react to changes in the palatability of the diet that have no influence on the food intake of nonobese rats. Addition of kaolin to the diet, which increases the food intake (in grams) of normal rats, caused the obese rats to reduce their food intake and lose body fat. This reaction depends, however, on the severity of the

hyperphagia in the obese rats, and unattractive diets can be used to differentiate between degrees of hypothalamic damage.

Reduction of food intake by water restriction, recently reported by Crampton & Lloyd (58), may involve both hunger—via concentration of metabolites affecting the hypothalamic regulator—and appetite. A restriction of 52 per cent of the water intake of rats reduced their food intake 27 per cent, their gain in weight 51 per cent, and the total efficiency of gain (gain/food) 31 per cent.

The modification of food intake through cortically mediated impulses does not usually have such pronounced effects as the more primitive hunger. Cases of deliberate starvation to death, regarded by Schopenhauer as the greatest victory of the mind over primitive urges, are rare. Cases of psychological interference with the urge to eat are, however, frequent. Children and soldiers use refusal to eat as a means of exerting their individuality in a field where their hunger for freedom can be given expression even by the partial suppression of their hunger for food. In many of those cases a change in the attitude of parents or commanding officers may influence food intake more than do changes in the dietary composition.

*Disturbance in control.*—Disturbances in the control of food intake may result from influences on the primitive hunger mechanism or on the cortical controlled modifier. Many pathological changes reduce appetite; most dietary deficiencies have this effect. The best known disturbance leading to increased food intake is the hyperphagia produced by hypothalamic lesions. That this amounts to a change in rather than an absence of control has been shown by Kennedy (59). The hypothalamic lesions which produce hyperphagia and obesity in rats do not impair the mobilization of the fat stores. Obese rats can mobilize fats from their depots as fast as they can store fat in these depots. The food intake of these hyperphagic rats is not higher than that of lactating normal rats and is lower than that of rats lactating at a low environmental temperature.

Hypothalamic lesions however, are, not the only condition that may produce obesity. Obesity, according to Mayer & Barrnett's (60) masterly written review, is a common symptom of a variety of pathologic abnormalities leading to disturbances of the regulaton of food intake.

## Calorigenic Effect of Food

In animals kept in an environment above the "critical temperature" food increases the metabolic rate. This effect is called specific dynamic effect by Rubner or heat increment by Armsby. McClymont (60) suggests the use of "specific dynamic effect" for the calorigenic effect of specific nutrients or metabolites and "heat increment" for the calorigenic effect of entire rations. The calorigenic effect of food is much greater in ruminants than in animals with a single stomach and the high heat increment in cattle seems to be greater the more roughage they have in their ration.

Recent investigations on rumen fermentation, especially by Barcroft

and his co-workers [Elsden & Phillipson (54)], and by Marston (61) and McClymont (63), indicated that low fatty acids, particularly acetic, propionic, and butyric acids, were practically the only compounds that carried carbon, and with it chemical energy, from carbohydrates to the bloodstream of ruminants.

Based on these findings, McClymont (62) has formulated a well-supported theory on the high calorigenic effect of food intake in ruminants. The argument for this theory goes as follows: (*a*) In ruminants, carbohydrates are not directly absorbed but fermented to fatty acids, $CO_2$ and $CH_4$. These fatty acids are absorbed from the rumen in great enough quantity that their oxidation accounts for the major part of ruminant heat production; (*b*) Acetic acid is the major part of the fatty acids formed in rumen fermentation; (*c*) Acetic acid is lipogenic but not glyconeogenic and is not utilized to any considerable extent in protein synthesis. The absorbed acetic acid therefore can only be used for fat synthesis or has to be oxidized; (*d*) All tissues can oxidize acetic acid but only a few, mainly liver, mammary gland, and adipose tissue, can utilize acetic acid for fat synthesis; (*e*) The relatively greater chance for oxidation than for use in fat synthesis explains the high specific dynamic effect of acetate directly observed by Lusk in the dog, and by Marston (63) in the sheep; (*f*) The high heat increment observed in ruminants can therefore be explained by the abundant formation of acetic acid with its high specific dynamic effect.

Morrison (64) measured the metabolic rate of nine adult female rats on *ad libitum* feed intake. The respiration apparatus, erroneously called a calorimeter, was kept in a thermostatat at 22° to 23°C., and the rats, by their heat production, raised the temperature in the chamber 2° to 3° above that of the thermostat; presumably, therefore, the rats were at a lower temperature, the lower their metabolic rate. A partial regression equation for metabolic rate and food intake revealed a calorigenic effect of 30 per cent of the food energy, but multiple regression equations, including body weight, led to a calorigenic effect of only 18 per cent. The temperature of the chamber was lower than the critical temperature of the fasting rat, which is 30°C. (49). Apparently the experiments were not planned originally for the measurement of calorigenic effect of food, and multiple regression equations are an inadequate substitute for proper original planning of experiments.

That in mice one can distinguish between "good feeders" and "bad feeders" as among farm animals and human beings has been demonstrated by Lyon, Dowling & Fenton (65). These workers showed that mice of two different strains consumed more food when fed a diet with 50 per cent fat than they did on a 5 per cent fat ration. However, there was a difference between the two strains' metabolic response to the increase in food intake. The mice of one strain, C57, increased their $O_2$ consumption with the increased food intake. Mice of the other strain, C3H, maintained the same metabolic rate at the two different levels of food intake and deposited more body fat when eating more food; these were the good feeders.

## Food Energy

The period of this report (1953–54) marks the centennial of Armsby's birth (see 66) and that of the Agricultural Experiment Station of Möckern-Leipzig. To celebrate the centennial of that station, the "Oskar Kellner Institute für Tierenährung" was founded in Möckern-Leipzig under the direction of Professor Nehring, and two centennial volumes have been published. One comprises the publication of the "Nachlass" of Kellner's work and that of his follower, G. Fingerling (67). The second volume contains contributions from representatives of various countries who work in the field of animal feed utilization. Armsby in the United States and Kellner [Breirem (68)] in Germany were the foremost pioneers in the application of the energy concept to the problem of feeding farm animals. Their work on net energy (Armsby) or dynamic energy (Kellner) as an expression of the useful effect of feeding and as a means of expressing nutritive content of feed is now classical and, to a certain extent, final.

With regard to practical application in feeding, Kellner's work has had much more success than Armsby's. Kellner's starch values are widely used in Europe. Armsby's net energy is taught in colleges in this country, and not always with the clarity it deserves, but the farmers and farm advisors use total digestible nutrients (TDN). This method of expressing nutritive content in one figure is based on the digestible energy to be sure, not quite correctly either [Maynard (69)], but not on Armsby's or Kellner's major achievement, net energy.

In this centennial volume, Möllgaard (70) still maintains that food values for dairy cows should be measured by difference trials in old steers so that only fat production results. Axelsson (71) expresses the opinion that the work of Kellner and of Armsby is wrong and that feeds should be measured by metabolizable energy instead of net energy. Breirem (72), however, concludes that net energy is the valid measurements of nutritive content. Crasemann (73) and his co-workers have measured net energy in smaller animals: sheep, chickens, and rabbits. In general they confirm net energy as a basis for feed evaluation. Fuchs (74) and Nehring (75) propose to measure "absolute" feed values by an abstract "Kellner Unit," 2,500 kcal. net energy, the equivalent for which in starch is different for different animals and different types of production.

Kleiber (76) feels that this abstract "absolute" Kellner unit is unnecessary since the unit of kcal. net energy exists already, and to express nutritive content in Fuchs' Kellner Units means to abandon Kellner's major advantage over Armsby, namely his use of starch as a well-defined reference substance, a reliable material basis for measurements. Recognizing the limitations of difference trials for measuring nutritive contents, Kleiber suggests replacement trials such as those used for decades in Scandinavia on a practical scale [Eskedal (77)]. He recognizes also the limitations of replacing a reference substance (such as starch or barley) against a feed to be investigated which has a different protein: energy ratio. Kleiber proposes to use for

replacement trial mixtures of at least two well-defined reference substances such as glucose, representing protein-free food energy and casein, representing food protein.

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# PHYSIOLOGICAL ASPECTS OF GENETICS[1,2]

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Since the appearance of "Physiological Aspects of Genetics" by Horowitz & Owen (74), many important pertinent topics have been reviewed. These include discussions or books on biochemical genetics (4, 66, 138), nucleo-cytoplasmic relations in microorganisms (45), viruses (40, 77, 93, 137), and genetics of microorganisms (20, 25, 108, 125, 135). Other reviews of more general interest include discussions by Stern (131), Demerec (38), and Muller (102). The excellent recent review of the genetics of microorganisms by Zelle (145) has covered many of the topics normally included within the scope of physiological genetics. The present reviewers have accordingly taken the liberty of limiting this discussion to an evaluation of some fundamental concepts concerning the nature of genetic mateial and its cellular function. In this presentation complete bibliographical coverage has not been attempted. Insofar as possible, references have been made to those papers most pertinent to the discussion, supplemented by recent reviews.

## THE CHEMICAL NATURE OF THE GENETIC MATERIAL

The conclusion that the genetic information is carried by deoxyribonucleic acid (DNA) has gained wide acceptance. This conclusion has been based on three main lines of evidence: (*a*) DNA, which is primarily localized in the nuclei of all plant, animal, and microbial cells which have been examined, is present in cells of any one organism as a function of ploidy (110). (*b*) DNA is apparently the specific agent responsible for transformation of specific heritable characteristics in certain strains of bacteria (75, 76). (*c*) DNA is apparently the carrier of genetic information in certain bacteriophages since the transfer of DNA rather than protein provides the link between parent and progeny particles (72).

The observation that the influenza virus (1, 2), like the plant viruses, contains ribonucleic acid (RNA) rather than DNA suggests that DNA is not the only molecular species responsible for genetic transfer. In view of the relationship between the genetic material and protein synthesis to be discussed below, corresponding or analogous specificity would be expected to reside at least in part in RNA and protein.

Marshak (96) has reported that the pronuclei of sea urchin eggs are devoid of DNA, based on the somewhat unsatisfactory grounds of visual estimation of Feulgen stainability of interphase nuclei, and on what would

[1] The survey of the literature pertaining to this review was completed in July, 1955.

[2] The following abbreviations are used in this review: DNA (deoxyribonucleic acid); RNA (ribonucleic acid).

appear an undue reliance on the sensitivity of the isotope dilution method.

*Structure of DNA.*—Current views on the structure of DNA are based essentially on detailed chemical analysis (26) and on x-ray diffraction studies (48, 143). These two lines of evidence have led Watson & Crick (140) to propose a structure in which two complementary polynucleotide chains are wound helically around a common axis, held together by hydrogen bonding between specific purine and pyrimidine components, with the chains having opposite polarity with respect to the sequence of phosphate-sugar linkages. Slight modifications in this basic structure which have subsequently been suggested include a decrease in the diameter of the molecule (47) and a shortening of the length of the individual polynucleotide strands through periodic, staggered, interruptions approximately 50 nucleotide units apart (36). However, the interpretation of denaturation studies, which led to the proposed periodic interruptions (36) and separation of the two strands (126), has been questioned (41).

The Watson & Crick structure of DNA has an advantage over previously proposed structures in that it provides a mechanism for the specific reduplication requisite for genetic material (141). In this structure the biological specificity, residing in the sequences of bases in each of the two complementary strands, can be duplicated by the assembly, from smaller nucleotide units, of complementary strands through breaking and reformation of hydrogen bonds.

An implication of the proposed structure is that all the material of the actual parental strands must be conserved during the reduplication process. Hershey (71) has shown by the use of radioactive isotopes that phosphorus and purine and pyrimidine carbon are retained in bacterial DNA during cell division. Most of the parental phage $P^{32}$ has been found to remain in high molecular weight DNA during phage multiplication (139), despite the losses which might be expected during such an infectious process.

The separation of the two coiled helices during reduplication would seem to raise serious problems. Delbrück (37) has suggested a mechanism involving breaking and joining at every half turn of the helix. Other possibilities proposed involve localized separation of the strands by transverse twisting (109) and conversion into a coiled system permitting complete separation (58, 91).

The functional diversity of genetic material would demand a corresponding diversity of composition in DNA. Although it is not yet possible to isolate DNA fractions corresponding to single functional genetic units, the heterogeneity of DNA from single sources has been demonstrated by a number of techniques including salt-fractionation (32, 33, 92), ion-exchange (11), and sedimentation (119).

It might be expected that the replacement in DNA of a specific base by an analogue would result in mutation or in failure of normal replication. The incorporation of halogenated uracil into DNA of *Escherichia coli* and of phages T2 and T5 leads only to inhibition of growth of the bacteria but not

to detectable mutation, but the more extensive replacement of thymine in phage, by these analogues, is associated with the formation of noninfective particles (42, 144). In view of these results, and those of Dunn & Smith (43) on the replacement of adenine by endogenous 6-methylaminopurine in *E. coli* DNA, it would appear that a certain degree of functional flexibility in the specific base content of DNA is possible, provided that incorporation is random.

Attention should be drawn to recent studies bearing on the enzymatic mechanism of nucleic acid synthesis. It would appear that nucleosides and nucleotides can be synthesized by a number of different enzymatic sequences either from free bases or from base precursors by reactions in which labilization by phosphate plays an important role [see (115)]. Phosphate is also involved in the enzymatic synthesis of high molecular weight polynucleotides from nucleoside diphosphates by an enzyme, polynucleotide phosphorylase, recently discovered by Grunberg-Manago & Ochoa (65).

*Structure of RNA.*—Evidence is now accumulating which suggests that RNA has structural similarities to DNA, as might be expected if the specificities of the two were related. In contrast to DNA, RNA has a more irregular, probably branched, but basically helical structure as indicated by x-ray diffraction studies (112, 113). RNA from animal sources tends to contain equimolar amounts of adenine and uracil, as well as of guanine and cytosine [(44) and data quoted in (113)]. However, this equivalence is not found in RNA from certain bacteria (44), certain plant viruses (113), and in strains of the influenza virus (2). An equivalence of base pairs would be expected in RNA if its structure were similar to that of DNA, and the apparent randomness and lack of base equivalence in bacteria and certain viruses makes it difficult at present to visualize the mechanism of RNA reduplication in terms of the DNA model.

*Size of the genetic unit.*—The ultimate goal of physiological genetics, the correlation of the structure of the genetic material with its function, requires a determination of the minimum size of the genetic unit. In recent years the concepts of the unit of mutation and the unit of crossing-over, originally identified with the functional unit, have been undergoing revisions. In many cases it has been found that both mutation and crossing-over are possible within the functional unit. This change in concept, derived from studies summarized in a symposium on pseudoallelism and the theory of the gene (60, 84, 87, 130, 133), has required a downward revision of the size of the minimum genetic unit.

The variety of phenotypic expressions and the differential selective effects of suppressors (142) on members of multiple allelic series suggest a correlation of functional heterogeneity with structural heterogeneity similar to that in pseudoallelic series (61, 83) (as defined in terms of rare crossover events). However, the functionally heterogeneous subunits must be related in that they all affect the same phenotypic character. The specific phenotypic expression of a complex locus therefore appears to be the result of the co-opera-

tive functioning of all of its subunits. This expression can be altered by mutation of any of the subunits or by changing their spatial relationships by crossing-over.

A study by Benzer (12) of the *r* mutants of bacteriophage T4 has shown that one of the loci responsible for rapid lysis is separable into two genetically discrete major functional subunits. Mutation at any one of a large number of points within each subunit, as determined by rare crossovers, results in expression of the *r* phenotype. Each of the two major subunits has been estimated in terms of the Watson & Crick model to consist of $4\times10^3$ nucleotide pairs, while the size of the unit of recombination was estimated to contain 12 nucleotide pairs. A corresponding analysis by Demerec *et al.* (39) of complex loci controlling nutritional requirements in *Salmonella* by means of transduction tests has shown that each can be subdivided into a large number of subunits. If recombination by means of transduction is equivalent to crossing-over, the size of the subunits is very small, perhaps of the same order suggested by Benzer (12) for phage. In *Salmonella* these complex loci appear to be in turn functional units of a larger complex controlling sequential biosynthetic steps (38a).

The close proximity in *Salmonella* of loci determining the different steps in the syntheses of cystine, of histidine, and of tryptophan, is striking in view of the extreme rarity of such relationships in other microorganisms and raises many interesting questions as to its evolutionary and functional significance. It is intriguing to postulate that a functional relationship may be involved within this higher complex, such as the association of the different enzymatic sites of activity with a common macromolecule or cellular structure in this organism. An analogous interpretation of the co-operation of the subunits of a locus concerned with a single heterocatalytic function would be that each of the subunits is responsible for the transmission of its specific portion of the total information required for synthesis of an effective enzyme. According to this concept it is possible to interpret position effects (87, 120, 121, 132) in terms of the effects of adjacent chromosomal material on the efficiency of transfer of information from the genetic to the functional material. Suppressor mutations (142) could be visualized as supplying information or material, at the genic level to the mutant locus the function of which is thereby restored or, at a synthetic level intermediate between gene and enzyme with restoration of the normal phenotype.

Although these concepts may be of some interpretive value, it should be pointed out that their application to the complicated but related effects of genes such as Activator, Dissociation, and the like in maize (7, 22, 94) is as yet obscure.

*Organization of the chromosome.*—The above views on the fine structure of the genetic material may prove to be naive when attempts are made to extend them to the more complex organizational level of the chromosome. However, it seems justified to attempt to correlate what is known about the divisibility of the genetic locus with the classical view of its discrete particulate nature.

It is known that the chromosome is composed of DNA, protein, and some RNA. Treatment with deoxyribonuclease removes DNA without loss of the structural identity of the chromosome, whereas proteolytic enzymes bring about chromosome fragmentation. Ultraviolet spectroscopy indicates the existence of localized areas of nucleic acid concentration. The evidence then suggests that the chromosome is made up of a continuum of protein with local concentrations of DNA. The correlation of these areas with genetic loci, one of the milestones of classical genetics, together with the behaviour of the gene as a discrete entity in crossing-over, reflects the discontinuous structure of the chromosome. Bernstein & Mazia (13, 14) have observed that the chromosomes of sea urchin sperm could be solubilized by treatment with chelating agents, which would be expected to break only salt linkages. Treatment of sea urchin sperm heads (13, 14) and of *Drosophila* salivary gland chromosomes (97) released macromolecular aggregates of nucleoprotein. The size of aggregates from salivary gland chromosomes approximated that of the chromosome bands. The solubilizing effect of chelating agents was interpreted by Mazia (97) as the removal of bivalent cations which bind the nucleoprotein aggregates together. This view of the structural function of bivalent cations is consistent with the earlier observation of Steffensen on the induction of chromosome breakage in *Tradescantia* by a magnesium deficiency (128), and the subsequent demonstration of a similar effect by a calcium deficiency (129). In an extension of the hypothesis it has been shown that the frequency of crossing-over in *Chlamydomonas* is significantly increased by treatment with ethylenediaminetetraacetic acid tetrasodium salt (Versene) (46). It is tempting to propose that the regularity of crossing-over of intact loci is related to those units delineated by salt linkages. Crossing-over would then take place by breakage and reformation of salt linkages between the intact loci. This hypothesis presupposes that breaks occur simultaneously in both homologous chromosomes.

A mechanical breakage hypothesis is beset by the obvious intrinsic difficulty of accounting for the extreme precision of exchange of homologous units. The cytological observation that a rather exact pairing of homologous chromosomes occurs during meiosis suggested to Belling in 1933 (10) that crossing-over occurs as a consequence of exchanges during reduplication. This view has been revived as a consequence of the increasing interest in this problem stimulated by the Watson & Crick DNA model. The problem, however, must be viewed from two aspects: (*a*) the replication of the genetic material, the locus, and (*b*) the replication of the chromosome. The model itself, and the Delbrück (37) proposal of a means of separating the strands, suggests that crossing-over occurs during and as a consequence of synchronous reduplication of the polynucleotide chains. This view has been used to account for crossing-over in bacteriophage by Levinthal (86) and could explain crossing-over within complex loci and during phage reproduction provided that in both cases exchanges involve single DNA strands. However, the accumulated evidence on polyteny and chromosome heterogeneity in higher organisms makes the application of this concept difficult at the chro-

mosomal level. An analogous hypothesis would involve exchange of replicated intact loci during the process of chromosomal assembly. This hypothesis, essentially that proposed by Belling (10), and recently restated and elaborated (85, 116), can provide a mechanism for the extrachromosomal transmission of genetic material involved in transformation and transduction. The incorporation of the DNA transforming agent and the phage vectored transducing material into the bacterial chromosome might occur during chromosomal assembly. Gene "conversion," first proposed by Lindegren (88, 89) to account for abnormal segregation in yeast, and subsequently encountered in *Neurospora* by Mitchell (99), essentially involves the transfer of genetic information from one chromosome to its homologue independently of adjacent loci, an event extremely improbable in terms of conventional reciprocal crossing-over mechanisms. It seems probable that gene conversion is analogous to transformation and transduction in that it might involve the extra reproduction of specific loci thus increasing the frequency of their incorporation during assembly.

*Summary.*—In summary, the genetic material must be envisioned as units of mutation, function, and crossing-over. The unit of mutation might involve only the sequence of two nucleotides along the DNA strand suggested by the Watson & Crick model. The functional unit, composed of a number of these mutable subunits, is concerned with the transmission of the sum of the information contained within the individual nucleotide sequences. The differentiation of this unit into subfunctional portions larger than the mutational units is indicated by the block-wise restoration of function in cases of position pseudoalleles. Crossing-over can apparently occur as a consequence of events either at the level of organization of the gene or at that of the chromosome.

## Gene Action

If the plausible assumption is made that the phenotype of an organism is determined by its enzymatic constitution, gene function must be interpreted in terms of the mechanism of transfer of information from the gene to the enzyme molecule. This raises the question whether the information transfer is direct or conveyed through an intermediate. Present evidence suggests that the microsomes are the major sites of protein synthesis in the cell (6). This implies an indirect transfer of information from the nucleus to the microsomes.

Considerable evidence has been amassed which implicates RNA in this intermediate role. As first shown by Caspersson & Schultz (24), cell growth and activity are associated with an increase in cytoplasmic ribonucleoprotein. This relationship has been confirmed and considerably extended by the use of radioactive isotopes and enzymatic and microsurgical techniques as extensively reviewed by Brachet (18).

*Role of the nucleus.*—Some protein synthesis has been demonstrated in isolated calf-thymus nuclei. The inhibition of synthesis by deoxyribonuclease

and its restoration by DNA implicates a DNA controlled mechanism (5). Although studies on *Acetabularia* and *Amoeba* have established that some protein synthesis can be attributed to the nucleus; cytoplasmic protein synthesis can proceed after enucleation (19, 98, 136). The equal incorporation of labelled carbon dioxide into the protein of nucleated and enucleated fragments of *Acetabularia* suggests that the role of the nucleus is minor in this case (19), in contrast to the estimated nuclear synthesis of 40 per cent of the total protein synthesized in *Amoeba* (98).

The lesser effect of the nucleus in *Acetabularia* has been tentatively suggested to be associated either with photosynthetic processes or with the genetic autonomy of the chloroplasts (98). In *Amoeba* the evidence strongly supports the view that the nucleus directly controls the enzymatic activities of the microsomal fraction (16) and the synthesis of RNA (78, 90). Furthermore, treatment of intact *Amoeba* with ribonuclease was found to inhibit cell division and protein synthesis. These inhibitions were reversed after the addition of yeast nucleic acid (17). The elegant enucleation studies when coupled with the mass of information on the association of RNA and protein turnover in diverse animal tissues [for review see (18)] amply support the general concept that RNA plays an intermediate role in the transfer of information from DNA in the nucleus to sites of protein synthesis in the cytoplasm.

*Nucleic acids and enzyme synthesis.*—Perhaps the most direct information on the role of RNA in protein synthesis has been derived from studies on the induced biosynthesis of specific enzymes in microorganisms. This subject has been repeatedly and exhaustively reviewed (3, 31, 51, 101, 124). Inducible enzyme systems constitute uniquely advantageous material for the study of the synthesis of specific proteins independent of cell growth. Furthermore, in microorganisms, enzyme synthesis can be initiated, followed, and terminated under precisely controlled experimental conditions.

The relationship of specific induced enzyme synthesis to amino acid assimilation and to over-all protein synthesis has been firmly established [for review see (51)]. These relationships have been derived from studies of the effects of antibiotics and enzyme inhibitors on amino acid incorporation into protein (55) and on enzyme synthesis (34, 55, 82).

Current views on the mechanism of enzyme induction involve the attachment of a specific inducing molecule, which is generally but not always a substrate, to a site in the cell other than a preformed enzyme molecule. The first definitive evidence for this concept was derived from the kinetic studies of Pollack on the induction of penicillinase by *Bacillus cereus*, in which each molecule of penicillin fixed led to the synthesis of at least 10 molecules of penicillinase (111). A similar conclusion was based on the considerable difference in the dissociation constants of $\alpha$-methylglucoside as an inducer and as an inhibitor of maltase activity in yeast (123). This conclusion is also supported by the increasing number of examples of enzyme substrates which are not inducers, and inducers which are not substrates. For example, thio-

methyl-β-D-galactoside, a nonsubstrate inducer of β-galactosidase, has been shown to be fixed by *E. coli* in proportion to the amount of enzyme synthesized, and to be displaced from its site of fixation by thio-phenyl-β-D-galactoside (27). The latter is not a substrate, is not an inducer, and is a competitive inhibitor both of induction and of enzyme activity. The association of β-galactosidase with an "enzyme-forming" site has been deduced from experiments on the specific activities of intact cells and cell extracts of *E. coli* during adaptation and deadaptation (15).

Swenson & Giese (134) demonstrated that high doses of ultraviolet light inhibited induced enzyme formation in yeast; the action spectrum for inhibition closely resembled the absorption spectrum of the nucleic acids (134a). It was subsequently shown by Kelner (79) that less intense irradiation selectively inhibited DNA synthesis, without interfering with normal protein and RNA synthesis. Similar results were obtained following the inhibition of DNA synthesis by mustard compounds (106, 107, 118).

Another useful approach has involved limiting nucleic acid synthesis by the use of purine and pyrimidine analogues and the use of purine and pyrimidine requiring mutant strains of bacteria. The purine analogue, 8-azaguanine, has been found to inhibit β-galactosidase synthesis in *Staphylococcus aureus* without affecting synthesis of RNA or DNA (35). However, the analogue was incorporated into RNA to a significant extent, and it was suggested that the RNA synthesized was rendered ineffective in protein synthesis. The pyrimidine analogue, 5-hydroxyuridine, inhibits β-galactosidase synthesis in *E. coli*, and a pyrimidine requiring mutant was unable to synthesize the enzyme in the absence of uracil (124). In contrast, a thymineless mutant of *E. coli* although unable to synthesize DNA in the absence of thymine, can synthesize RNA and xylose isomerase under these conditions. Growth of this mutant in the absence of thymine results in "unbalanced growth" and death (8, 29, 30).

The most definitive new evidence on the participation of RNA and DNA in enzyme synthesis has come from studies on protein and nucleic acid synthesis by disrupted preparations and lysozyme treated cells (9, 52, 95). The elegant studies of Gale & Folkes have established that the removal of nucleic acid from disrupted cell preparations of *S. aureus* decreased the incorporation of labelled amino acids into protein and decreased synthesis of catalase, β-galactosidase, and "glucozymase." In the presence of a mixture of amino acids, hexosediphosphate, and adenosinetriphosphate protein synthesis is stimulated by nucleic acid preparations (52). The correlation between the extent of nucleic acid depletion and the effectiveness of restoration of protein and enzyme synthesis by RNA, DNA, purines, and pyrimidines, and combinations thereof, differs for the synthesis of the various enzymes studied (53). It appears that the extraction methods used remove RNA more rapidly than DNA and that reactivation by DNA can be demonstrated for catalase and glucozymase only after the DNA concentration has been lowered to a level limiting synthesis. In the case of β-galactosidase only purines and py-

rimidines were stimulatory for enzyme synthesis at intermediate stages of nucleic acid depletion. More depleted preparations, the activity of which is sensitive to deoxyribonuclease and ribonuclease, responded to purines and pyrimidines, and to DNA, but not to RNA. The most striking enzyme synthesis was observed in the presence of both purines and pyrimidines and DNA. The data suggest a synergistic effect of this combination. The inactivity of RNA obtained from induced cells was interpreted as indicating that the RNA concerned with β-galactosidase synthesis is unstable and that active synthesis of RNA is essential. It is intriguing to postulate that RNA stability differentiates constitutive and inducible enzyme systems.

Di- and tri-nucleotides derived from *Staphylococcus* RNA by partial enzymatic hydrolysis, showed up to 100 times the activity of intact RNA in stimulating the incorporation of amino acids into protein. Different polynucleotides differentially stimulated the incorporation of specific amino acids (54). Stimulation of incorporation by intact RNA is species specific (52). Gale & Folkes (53), therefore, propose that incorporation in species specific proteins depends upon specific sequences of nucleotides in the RNA. This again suggests involvement of an RNA template in protein synthesis. In light of these observations, it would appear that DNA may determine the nature of this RNA template. The structural specificity of the DNA responsible is indicated by the finding that in the restoration of the synthesis of β-galactosidase by deoxyribonuclease treated disrupted preparations, DNA from induced cells is three to four times as effective as DNA from uninduced cells (53).

Halvorson *et al.* (67 to 70) have shown that the material necessary for enzyme synthesis in yeast is primarily derived from the free amino acid pool. With *E. coli*, which lacks a significant amino acid pool, it has been demonstrated that enzyme protein is synthesized *de novo* without any detectable participation of preformed or precursor protein material (73, 114).

It would seem, therefore, that an hypothesis involving a specifically directed assembly of individual amino acids or small peptides is consistent with the information thus far available. The nature of the template responsible for this orderly assembly has not been definitively established, however, all available evidence points to the nucleic acids or nucleoproteins. Since the structure of RNA is by no means clearly understood, various investigators have attempted to correlate the sequences of bases in the Watson & Crick structure of DNA with amino acid sequences in proteins. In one such hypothesis the sequence of the 20 possible combinations of three nucleotides in the double helix determines the sequence of assembly of the 20 amino acids into protein (56, 57). Another hypothesis involves the assumption that orderly assembly of amino acids is primarily oriented by the spacing of aromatic amino acids as determined by specific pairs of bases (117). It has also been suggested that the presence of short polynucleotide branches on the main structure and the accessibility of free amino groups may be determining factors in the biological activity of DNA (47).

It is well established that biologically functional DNA is usually associated with protein. Feughelman *et al.* (47) have shown that x-ray diffraction patterns of deoxyribonucleoprotein fibers have essentially the same characteristics as those of intact sperm heads and of T2 and T4 bacteriophage. Whether nucleic acids act as such or as nucleoprotein remains to be established.

*Summary.*—The information summarized in the previous section establishes with some degree of certainty that both DNA and RNA possess some degree of genetic autonomy. Furthermore, DNA and RNA and protein possess a high degree of chemical specificity. The obligatory relation between RNA turnover and protein synthesis in almost every instance studied, the sensitivity of protein synthesis to ribonuclease, and the independence of RNA and protein synthesis of nuclear DNA in *Amoeba* and *Acetabularia*, suggest that the relation between RNA and protein synthesis is direct. The stimulation of protein synthesis by DNA in disrupted preparations of *Staphylococcus* and its sensitivity to ribonuclease indicate an indirect relation between DNA and protein. In view of this it seems reasonable to propose that the primary heterocatalytic function of DNA, acting either alone or in combination with protein, is to determine the structural specificity of RNA by serving as a template for its initial synthesis. RNA, the structure of which appears to be similar to, but more heterogeneous than, DNA, may correspondingly serve as a template for the assembly of amino acids or small peptides into proteins.

The genetic autonomy of RNA is indicated by the presence of only ribonucleoprotein in plant viruses and by the demonstration that its turnover is independent of DNA. In terms of a unitary hypothesis, the plant viruses can be visualized as transmitting the specific protein determining material, RNA, required for virus reduplication in the host cell. In contrast, bacteriophages, and the animal viruses with the exception of the influenza virus, transmit information leading to the formation of the specific protein synthesizing templates.

Such a unitary hypothesis, though consistent with the information thus thus far presented, cannot readily account for the apparent complete absence of RNA turnover during T2 and T4 bacteriophage reproduction as determined by phosphorus incorporation (28). It is possible that during phage reproduction DNA can determine protein synthesis without involving RNA. However, host cell RNA in bacteriophage infected systems might conceivably be reorganized at the nucleotide or polynucleotide level without detectable phosphorus exchange.

Too little definitive information is available to permit more than speculation on the role of RNA in protein synthesis in the nucleus. Since RNA is apparently present in all nuclei, it may play similar roles in both nuclear and cytoplasmic protein syntheses. Alternatively, DNA may determine protein synthesis in the nucleus without the participation of RNA, as suggested for bacteriophage reduplication. There is no a priori reason to believe that the functional properties of RNA and DNA are necessarily distinct. Their struc-

tural similarity suggests that they may possess overlapping functions, both genetically and catalytically.

## Genes and Differentiation

It would be presumptuous and superfluous to attempt to cover the extensive and comprehensive studies on growth and differentiation in the short space available here. However, physiological genetics cannot be adequately reviewed without allusion to the relationship of the genetic material to the orderly but complex processes leading to the fabrication of the individual. Consistent with the general approach developed in the preceding sections, the reviewers have preferred not to be narrowly restricted by available data.

In this discussion we would like to revivify an hypothesis first presented by Monod (100) and extended by Spiegelman (122). This hypothesis suggests that intracellular and extracellular influences evoke or suppress the synthesis of specific enzymes affecting the molecular constitution of the cell, thus leading to differentiation. The plasticity of the enzyme complement of the cell, as revealed by studies on the induced synthesis of enzymes, offers a means of correlating development with molecular interrelationships.

In a developing embryo, those gradients established as a result of growth and cell multiplication could result in quantitative changes of enzymatic reactions. As a consequence of inhibitions or stimulations, products might be accumulated which would induce the synthesis of enzymes, conferring new functional activities on the cell. For example, if, in an enzyme-catalyzed sequential conversion of substance A through B to C, the conversion of B to C is inhibited, the accumulation of B will induce the formation of a new enzyme catalyzing the conversion of B to X. The accumulation of X could then induce the synthesis of another enzyme capable of converting substance X to Y, and so on in a manner analogous to sequential adaptation (induction) (127). As indicated in the preceding section an inducer, such as compound B, need not be a substrate for the induced enzyme. Thus the accumulation of B might lead to the synthesis of an enzyme acting upon a compound produced by an unrelated series of biochemical reactions and thereby lead to profound enzymological differentiation. Similar consequences can be envisaged in cases of stimulation of a specific reaction.

So far as is known to the reviewers there is no extensively studied example in the literature of the induction of such enzymological differentiation. However, an example is known in *Neurospora* in which the blocking of a specific reaction as the result of mutation results in the establishment of a new enzymatic sequence.

A mutant strain blocked in the conversion of dehydroshikimic acid to shikimic acid was found to accumulate protocatechuic acid, presumably derived from dehydroshikimic acid. Characteristically, the mutant strain metabolized the accumulated protocatechuic acid via protocatechuic acid oxidase. This enzyme is not normally present in wild type strains, but its synthesis can be induced by the addition of protocatechuic acid or an induc-

ing analogue (63). Thus a mutation has the appearance of resulting in the production of a new enzyme as a consequence of an impaired enzymatic function.

In addition to the oxidative conversion of protocatechuic acid to $\beta$-ketoadipic acid (62), protocatechuic acid gives rise by an alternative pathway to a brown melanogenous pigment (64). The activity of protocatechuic acid oxidase in the mutant strain as well as in the induced wild type strain is suppressed by the presence of an alternate carbon source (59) in a manner reminiscent of diauxie (100). Thus environmental conditions affecting the production or metabolism of protocatechuic acid can lead to qualitatively and quantitatively different morphogenetic characteristics.

Many other observations have been made on microorganisms implicating environmentally induced enzymatic modifications. These include the effect of bicarbonate on the accumulation of $\alpha$-ketoglutarate which in turn appears to influence the initiation and maintenance of melanin biosynthesis in *Blastocladiella emersonii* (23). A close relationship between inositol-phospholipide metabolism and morphogenesis has been observed in *Neurospora* (50). Genetic interference with inositol biosynthesis, or inhibition of efficient utilization of inositol by the gamma isomer of hexachlorocyclohexane, results in altered morphology (49).

The examples cited above indicate a few of the many possible mechanisms by which the enzymatic constitution of an organism can be affected by environmental and genetic events. An extension of these concepts and methodological approaches, used extensively with microorganisms, to the study of morphogenesis in more complex forms should prove fruitful, especially in view of the fact that a number of enzymes are known to be inducible in vertebrates (81).

Morphogenesis is too complicated a phenomenon to be explained as yet in terms of a single hypothesis. The important observations on nuclear transplantation by King & Briggs (21, 80) suggest a degree of nuclear differentiation as early as gastrulation in frog embryos. The specific morphogenetic effects of nucleic acid on salamander gastrula ectoderm in tissue culture, discovered by Niu & Twitty (103, 105), are suggestive of specific effects of different molecular species of nucleic acid on cellular differentiation. Although the identity of the active principle has not been definitively established, it seems clear that the developmental responses differ with the physiological state of the tissue of origin. If the active principle proves to be RNA, as seems to be indicated (104), its role might be analogous to that of RNA as described in the preceding sections; that is, it might determine the synthesis of specific enzymes leading to differentiation.

If the gene seems to have gotten lost somewhere in the foregoing discussion, it is because its function in differentiation cannot be directly ascertained. It would seem to be a truism to say that the enzymatic potentialities of the cell are gene determined; however, the specific enzymatic pattern imposed upon differentiated cells seems to be attributable to extragenetic fac-

tors. Unless we assume, as some have, that the process of differentiation is controlled by directed mutations or by specific genes determining the time and rate at which gene-governed reactions will occur, we are forced to look for mechanisms resulting in regular changes in patterns of enzymatic reactions within the framework of a constant genotype.

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# THE PHYSIOLOGY OF CONNECTIVE TISSUE[1,2]

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The realization that the connective tissues may be of importance in a number of physiological and pathological reactions has stimulated increasing investigative effort. The connective tissues are widely dispersed and exhibit marked structural and functional specialization. Their delineation is difficult, particularly with respect to physiological and anatomical differentiation from vascular structures.

It is necessary to limit the scope of this review both with respect to subject matter and extent to which individual contributions can be discussed. Since previous reviews in this series have been primarily concerned with biological aspects of the subject, emphasis will be placed on the chemistry, metabolism, and physical chemistry of extracellular connective tissue components. Several valuable monographs have appeared pertinent to the subject matter under discussion. Among these is a compilation of chapters on a variety of topics dealing with connective tissue which has been edited by Asboe-Hansen (2), a compilation of papers presented at a symposium on collagen edited by Randall (173), and the Transactions of the Josiah Macy Conferences on Connective Tissues (115). Valuable information is also contained in a monograph by Kent & Whitehouse (120). Reviews concerned with specific topics under discussion will be indicated below.

## Fibers

*Collagen.*—Collagen can be simply defined according to Gross (78) as "that class of fibrous proteins characterized structurally by a particular wide angle x-ray diffraction pattern, and in most, if not all, native species, a distinctive axial periodicity of about 640 Å, and chemically by high content of hydroxyproline, proline, glycine, and low moieties of aromatic amino acids." All of the above criteria are met by collagen from marine sponges, coelenterates, echinoderms, fishes, and mammals. Definitions and terminology in current usage relative to "native" collagen and its solubilization and degradation products, however, are in a confusing state with the major emphasis on differences in chemical and physical fine structure. Data have been obtained (220) to lend further support to the view (165) that intact hide collagen may be composed of a series of related proteins of not quite identical composition.

In spite of important advances, no completely satisfactory structure for collagen has yet been found. Various helical models have been recently pro-

[1] This review generally covers the period of June, 1954 to June, 1955, except for some sections which have been more extensively treated.

[2] The following abbreviations are used in this chapter: CSA (chondroitinsulfuric acid); HA (hyaluronic acid).

[3] Established Investigator of the American Heart Association, Inc.

posed (41, 97, 172, 175). Evidence has accumulated that the collagen fibril is made up of an aggregation of smaller units. Gross *et al.* (79) have postulated the existence of a very thin "tropocollagen" particle, having lengths in the long-spacing range, primarily from 1800 to 2300 Å. Small angle x-ray scattering has shown the existence of a sub-unit of dimensions 220 Å along the fiber axis and 40 to 90 Å at right angles (161). A structural sub-unit of 32.8 Å is indicated in diffraction patterns of both wet and dry fibers (233). In confirmation of Wyckoff's observations, Kennedy (119) has demonstrated a distinctly tubular appearance of collagen fibrils when sections are viewed in the electron microscope. A possible objection is that such hollow structures would require a very low density for collagen (174). According to Gustavson (83), the differences in the hydrothermal stability between fish and mammalian collagens are related to large differences in hydroxyproline contents and to different degrees of interchain hydrogen bonding between hydroxy and keto-imide groups. It is suggested that the strong cross-link common to all collagens may be an ester bond. Evidence for the existence of ester bonds in procollagen has been reported (82). The least degraded collagen preparation appears to be that which yields the lowest intrinsic free energy for dye binding (219).

On the basis of histological evidence, it has been suggested (216) that collagen is a polyphase system composed mainly of "collastromin" (consisting of a protein-mucopolysaccharide complex and an argyrophilic, fibrillar protein) and procollagen. However, it is inferred from studies on tendon that the collagen fiber is built up from at least two different protein components, neither of which corresponds to chondromucoid (CSA-protein complex) (10).

Further investigations of the molecular properties of soluble collagen have been conducted. Procollagen of mammal skin was found to be molecularly polydisperse at 30° with M averages from 74,000 (number average) to $10^7$ (weight average). It was suggested that procollagen solutions are temperature-sensitive reversible systems of aggregates (146). Acidic solutions of ichthyocol can be reconstituted into typical collagen-like fibrils. They contain long thin filamentous molecules (protofibrils) of a particle weight of one to two million, containing about one amino acid residue per Å of contour length and intermediate in shape between a random coil and a rigid rod (71). When these solutions are maintained at 30°, the optical rotation and intrinsic viscosity falls rapidly, leading to formation of a "parent gelatin" of M about 70,000 (72). By contrast, solutions of mammalian procollagen at this temperature are relatively stable in optical rotation and maintain a high intrinsic viscosity (141). Under special conditions Boedtker & Doty (23) obtained citrate solutions of ichthyocol with a narrow particle size distribution. The molecular units were rigid, rod-shaped particles of diameter 14 Å (hydrated); length 2900 Å; weight 300,000. It was suggested that this particle be called tropocollagen since it had the essential characteristics of the previously postulated building unit (79). Hide collagen extracted at 60° yields new "soluble collagen" intermediates in the collagen-gelatin transformation. Particles range in M from $1 \times 10^6$ to $20 \times 10^6$ and behave as stiff coils of three laterally

aggregated helix structures. The stability of these units at 40° is in marked contrast to the rapid degradation observed in solutions of procollagen and of ichthyocol at this temperature (221).

Samples of moderately high M (about $1\times10^5$) gelatin are found to be molecularly dispersed at 40° as random coils with mean configurations comparable to those of typical synthetic polymers. At lower temperatures and below a critical concentration for gel formation, aggregation occurs, leading to very small cross-linked crystallites (22). Similar conclusions as to molecular configuration of gelatin are drawn by other workers (76). An unusually high M of $6.0\times10^5$ found for a gelatin is apparently not due to the presence of dissociable aggregates (76).

The N-terminal residues of intact gelatins, predominantly glycine, are readily detected (38, 39). However, no N-terminal residues have been found in collagen (27) or procollagen (82). The amino acid composition deduced from the titration curve of gelatin has been shown to agree with conclusions from chromatographic analysis (118). Data concerning peptide sequences in gelatin have been obtained (188). Proteolysis of gelatin occurs by random breaking of peptide bonds as in thermal degradation and also by a specific bond breaking action (40). Collagen dissolves in anhydrous hydrogen fluoride (117) with denaturation (56). High pressures (3000 atm.) affect procollagen and gelatin solutions differently (215).

It is generally accepted that the cell has a major role in fibrogenesis. However, the level of molecular and structural development reached within the cell before secretion or extrusion of fibril precursors into the ground substance is unknown. Unstriated filaments, varying from 120 Å to 500 Å in length have been observed within the cytoplasm of fibroblasts, osteoblasts, osteocytes, and chondroblasts of embryonic tissue (102, 175). It has not been determined whether the filaments are collagenous, although evidence has been presented that these intracytoplasmic filaments are concerned with fibrogenesis (104). Apparently similar filaments have been observed within fibroblasts in the regenerating tendon of the rat (222).

Fibrogenic cells have been shown to contain cytoplasmic granules which contain polysaccharide, possibly acidic, as a principal component and protein with the characteristic amino acid composition of collagen. It has been suggested that the granules may secrete ground substance, synthesize the collagen fibril, or contain precursors of extracellular material (103, 175).

Collagen extracted from connective tissue by neutral salt solutions precipitates as typical cross-striated fibrils by raising the temperatures to 37°C. It is suggested that such soluble collagen exists in the ground substance, and, in the form of the tropocollagen monomer, may be a precursor in physiological fibrogenesis (80). The physical processes involved in fibrogenesis have been discussed by Randall (175). Thermally shrunk collagen is hydrolyzed 10 times faster by elastase than is elastin itself. It is suggested that both collagen and elastin contain the same elastase-susceptible elements but that collagen fibrils are surrounded by an elastase-resistant and heat-denaturable mucopolysaccharide-like envelope (9).

Slack (195) concludes that collagen synthesis in the rat may continue at a normal rate in an atrophying limb although he later finds (196) an increased rate of metabolism of one or more sulfated mucopolysaccharides in the tissues in general, and, in particular, in the atrophying limb. Scurvy does not accompany avitaminosis-A in the rat since the collagen concentration of new repair tissue is the same in normal rats, vitamin-A-deficient rats, and in vitamin-A-deficient rats given ascorbic acid (177). The rates of development of collagen and actomycin in the chick embryo are similar. The finding of increased amounts of NaOH-extractable collagen during periods of more rapid accumulation of collagen suggests that this material may be a precursor form of collagen (93). Hormonal regulation of collagen formation is indicated (99) by the finding that $\alpha$-estradiol benzoate, but not progesterone, markedly increases the uterine content of collagen.

Of some 25 proteins examined, only in collagen was hydroxylysine detectable (90). This amino acid is formed *in vivo* from lysine (192); its diastereoisomers have been resolved by ion-exchange chromatography (89). Little & Windrum (132) find that kidney reticulin shows a diffraction pattern typical of collagen, together with some additional rings due to a mixture of fatty acids. Reticulin of rat spleen is reported to bind insulin (33).

*Elastin.*—The histochemical similarities between the elastic fibers of the human common carotid artery and the ligamentum nuchae of the ox are reviewed (8). The staining reaction between orcein and elastin is due to formation of hydrogen bonds (223). Existing methods for the preparation of elastin may fail to remove associated proteins before causing degradation of the elastin. In addition, elastic tissue preparations differ from each other in relative concentrations of amorphous and fibrillar components (87). The situation is further complicated by the finding that elastase consists of two enzymes which act specifically on the two components of elastic tissue preparations (88). The properties of the grain membrane of cattle hide appear to be very similar to those of elastin (96). Wood (230) has postulated stabilizing cross-links for elastic tissue, but these remain to be demonstrated. Acid mucopolysaccharides appear to be important in determining the tensile properties of native tissue.

In the adult rat aorta, elastin, in common with the major portion of collagen, has a very slow rate of turnover (194). Elastase treatment of skin sections in a case of pseudo-xanthoma elasticum digested both the elastic tissue around pathological nodules and the degenerate masses in the nodules without attacking the collagen, and removed areas of colloid change, elastic fibers, and elacin in cases of senile elastosis, and the colloid of a colloid milium patient (69).

## Ground Substance

*Composition of ground substance.*—Information concerning the composition of the amorphous matrix of connective tissues, although still deficient, has increased markedly. It is apparent that this compartment must contain substances in transit between the vascular system and parenchymal

cells as well as those components peculiar to connective tissue. Relatively little progress has yet been made concerning the character of protein components and the nature of their interaction with polysaccharides. That certain proteins similar in nature to those present in blood serum are present in ground substance is apparent from studies of synovial fluid (181). The presence of soluble forms of collagen which may be precursors of collagen fibers has been emphasized by a number of investigators and has been discussed above. No soluble precursor of elastin has yet been demonstrated.

Several investigators (37, 191, 232) have found evidence of the presence of a protein, richer in tyrosine than is collagen, that is bound to mucopolysaccharides. Shatton & Schubert (191) have emphasized the possibility that CSA in cartilage is tightly bound to such a protein in a "mucoprotein." It is possible that both the qualitative and quantitative protein pattern may differ in specialized connective tissue.

There is increasing evidence for the existence of a multiplicity of mucopolysaccharides in connective tissue. The complexity of this system has been emphasized by Meyer (155) who has reviewed the properties of the following six different mucopolysaccharides which have already been demonstrated in connective tissue: (*a*) HA, (*b*) CSA-A, (*c*) CSA-B, (*d*) CSA-C, (*e*) chondroitin, (*f*) keratosulfate.

Evidence has accumulated suggesting the presence of more than one CSA. Such a possibility was originally suggested by the observation of Meyer & Chaffee (150) of the presence in pig skin of a polysaccharide with the previously established composition of CSA, but distinguished from that isolated from cartilage by a higher optical rotation and different solubility. In a subsequent investigation Meyer & Rapport (151) found evidence of this compound in tendon, heart valve, and aorta, and pointed out its resistance to testicular hyaluronidase. Further evidence for the existence of this compound was provided by Marbet & Winterstein (139) who isolated from beef lung a similar compound, named $\beta$-heparin, which had anticoagulant properties. Yamashina (234) confirmed these findings and found this substance had considerable anticoagulant potency by the thrombin method of Studer & Winterstein (206), but little activity by other methods. It was postulated that the mechanisms of action of "$\beta$-heparin" differs from that of heparin and heparin monosulfuric acid. What appears to be a similar substance has been isolated from gastric mucosa by Smith & Gallop (197). Schiller *et al.* (183) found evidence that the CSA of rabbit skin behaved as a mixture of two compounds, probably CSA-A and CSA-B, in the terminology of Meyer (155). This was evidenced by partial hydrolysis by testicular hyaluronidase as well as the previously reported low carbazole values and high negative rotation. Meyer *et al.* (156) have reported the isolation of CSA-B from ox ligamentum nuchae and state that it is considerably more labile to acid hydrolysis than is CSA-A and contains a different repeating unit than the latter.

Meyer (155) has also differentiated a third CSA (CSA-C) which is said to be present in umbilical cord, tendon, heart valves, and aorta, as well as in

hyaline cartilage. In contrast to CSA-B, but like CSA-A, it is hydrolyzed by testicular hyaluronidase. The solubility of the calcium salt in aqueous alcohol is greater than that of CSA-A and it has a somewhat lower negative optical rotation. It is suggested that CSA-C may be a somewhat lower M homologue of CSA-A.

On the basis of infra-red spectroscopic studies, Orr (166) has recently claimed that CSA obtained from tracheal cartilage is a mixture of two isomers, partial separation of which was claimed by countercurrent electrophoresis. It is suggested that these two substances may differ with respect to the position of the sulfate group. Davidson & Meyer (47) have isolated from cornea a fraction low in sulfate, but which contains galactosamine. This compound, which was named chondroitin, was shown to be hydrolyzed by both testicular and pneumococcal hyaluronidase and, on acid hydrolysis, yielded a disaccharide which is identical in infra-red spectrum with chondrosin prepared from CSA-A.

Meyer *et al.* (154) have reported the isolation from cornea of a mucopolysaccharide fraction which has little uronic acid but contains equimolecular amounts of glucosamine, galactose, and sulfate. This was named keratosulfate. This is in accord with the previous finding of galactose in a mucopolysaccharide fraction isolated from cornea by Woodin (231). The presence of a similar compound in human subcutaneous tissue has been suggested by Consden & Bird (37), while Gardell & Rastgeldi (73) have found evidence of this compound in human intervertebral discs.

Deiss & Leon (50) have isolated from bovine heart valves a fraction containing approximately equimolecular amounts of nitrogen, acetyl, hexosamine, hexuronic acid, and sulfate. No attempt was made to identify the hexosamine present. It was conluded that this substance is identical to hyaluronosulfate previously thought to be present in cornea by Meyer & Chaffee (149), but the existence of which has been recently denied by Meyer (153). No positive identification of components was undertaken in this study.

*Structure of mucopolysaccharides.*—During the past several years considerable progress has been made toward the elucidation of the structure of HA and CSA as well as the mechanism of action of hyaluronidase on these compounds. These advances have come about largely as a result of the efforts of Meyer & co-workers. The long accepted structure of the disaccharide, chondrosin, produced on the hydrolysis of cartilage CSA, has been shown to be fallacious (48). The proposal by Levene (128) that this compound is a galactosaminidoglucuronic acid, which was accepted by subsequent workers as a basis for structural studies on CSA (229), is not consistent with the finding originally reported by Masamune *et al.* (140) that this compound gives a positive Elson-Morgan reaction, indicating that the free reducing group is that of the glucosamine moiety. This discovery makes necessary a revision of the interpretation of previous studies on the structure of CSA. Davidson & Meyer (48) suggest that chondrosin is $\beta$-1,3,-D-glucuronidochondrosamine. The 1,3 linkage is suggested by analogy with HA (*vide infra*).

Studies on the mechanism of action of hyaluronidase has contributed materially to an understanding of the structure of HA. Weissmann *et al.*

(226) have shown that the action of testicular hyaluronidase on HA results in the formation of a homologous series of oligosaccharides which are thought to be polymers of the basic disaccharide unit, hyalobiuronic acid. The tetrasaccharide was found to be the predominant product, probably as a result of a transglycosidase action of hyaluronidase (224). Acid hydrolysis of the products of enzymatic digestion results in the production of a disaccharide isolated in a 60 per cent yield. The stucture of this disaccharide, hyalobiuronic acid, has been established by Weissmann & Meyer (225) as 3-O-(β-D-glucopyranosyluronic acid)-2-amino-2-2-deoxy-D-glucose as a result of their elegant structural investigations. The nature of the other glucosidic linkage is not yet established.

That bacterial hyaluronidases have a different mechanism of action than does the testicular enzyme has been suspected for some time, but no reasonable explanation was available until the recent finding by Linker & Meyer (130) of the formation of an unusual unsaturated disaccharide thought to be identical with hyalobiuronic acid except for a double bond in the 4,5 position of the uronic acid portion of the molecule.

Linker, Meyer & Weissmann (131) found that β-glucuronidase liberates glucuronic acid from a tetrasaccharide and a hexasaccharide obtained by the action of testicular hyaluronidase on HA. This action resulted in the production of a trisaccharide and a pentasaccharide, respectively. Further action of a crude testicular enzyme resulted in the release of N-acetylglucosamine (isolated for the first time from HA) and a disaccharide (identical with hyalobiuronic acid) from the trisaccharide, and a tetrasaccharide and N-acetylglucosamine from the pentasaccharide. No such result was observed as a result of the action of emulsin or bacterial β-glucosaminidase which have been shown to split N-acetyl phenyl-β-D-glucosaminide. No monosaccharides were obtained as a result of the action of the various enzymes on the disaccharide.

*Metabolism of mucopolysaccharides.*—Although information regarding the metabolism of the mucopolysaccharides of ground substance is still fragmentary, considerable progress has occurred during the past year. Some aspects of this field have been reviewed (55).

The discovery of a series of uridine nucleotides which contain the monosaccharide components of the mucopolysaccharides has led a number of investigators to suggest that these compounds may play a role in the biosynthesis of mucopolysaccharides. Park (168, 169) has demonstrated a series of amino sugar-containing uridine nucleotides in staphylococci, and Cabib *et al.* (31) isolated uridine diphospho-acetylglucosamine from yeast, while Smith & Mills (198) and Hurlbert & Potter (98) have isolated an apparently identical compound from liver. Pontis Videla (171) has found evidence of uridine diphosphogalactosamine in liver. Of especial interest is the preliminary report of Strominger (205) demonstrating the presence of uridine diphosphate acetylglucosamine phosphate and uridine diphosphate acetylgalactosamine sulfate in extracts of oviducts. It is suggested that the latter compound may be an intermediate in the synthesis of CSA.

The relationship of uridine nucleotides to uronide synthesis has been

clarified by the demonstration by Dutton & Storey (58, 203) of the participation of uridine diphosphoglucuronic acid in uronide synthesis. These findings have been confirmed and extended by Smith & Mills (198) and by Isselbacker & Axelrod (100). The isolation of an enzyme which oxidizes uridine diphosphoglucose to uridine diphosphoglucuronic acid has been reported (204).

That uridine nucleotides participate in the synthesis of disaccharides has been demonstrated by the finding by Leloir and co-workers (124, 126, 127) of the participation of uridine diphosphoglucose in the synthesis of trehalose phosphate, sucrose, and sucrose phosphate.

Considerable progress has been made in the understanding of the metabolism of hexosamines. The earlier finding by Lowther & Rogers (134) that the amide nitrogen of glutamine is the source of the amino group of glucosamine has been confirmed and extended (135) by the demonstration of the incorporation of $N^{15}$ of glutamine in the hexosamine of HA produced by streptococci and the demonstration of hexosamine synthesis in a cell free preparation of streptococci. This is in accord with the report of Leloir & Cardini (125) of the formation of glucosamine-6-phosphate from hexose phosphate and glutamine in the presence of an enzyme preparation obtained from *Neurospora crassa*. More recently Blumenthal *et al.* (19) have purified this enzyme and suggested that fructose-6-phosphate is actually involved in this transformation. An enzyme which brings about the N-acetylation of glucosamine-6-phosphate has been partially purified from yeast by Brown (30).

The *in vitro* synthesis of small amounts of hexosamine by subcutaneous tissue has been reported (24). Dorfman *et al.* (53) have demonstrated that glucosamine and acetate are utilized for HA synthesis by a strain of Group A streptococci, but N-acetylglucosamine is probably not utilized.

A number of studies continue to appear dealing with the utilization of radioactive sulfate for the study of mucopolysaccharide metabolism. Earlier studies along these lines have been reviewed by Boström (25), Dorfman (55), and Boström & Jorpes (26). Dziewiatkowski (59) has studied the effects of age on the incorporation of radioactive sulfate in the mucopolysaccharide fractions of skeleton, viscera, and pelts of rats. Evidence was found of greater incorporation of radioactivity in the fractions obtained from the younger animals. The incorporation of radioactive sulfate in the tissues of chick embryos and young adult rats and mice has been studied by Mancini & de Lustig (138). Much less accumulation of radioactive sulfate was observed in the parenchymatous tissues of adults than in those of embryos. A detailed study of the distribution of radioactive sulfate in the connective tissues of rats has been carried out by Davies & Young (49) and by Belanger (13). The latter author demonstrated that the material which contains the radioactive sulfate in bone and dentine is hydrolyzed by hyaluronidase except at the tendon insertion of bone. Upton *et al.* (217) have studied the incorporation of radioactive sulfate in healing wounds of normal and scorbutic animals and found maximal incorporation in the areas of most active collagen formation and minimal incorporation in the vicinity of immature fibroblasts and the relatively afibrillar scorbutic tissue. A decrease in the uptake of radioac-

tive sulfate by mast cells in tumors (produced by treatment with 9,10-dimethyl-1,2-benzanthracene) as a result of treatment with cortisone has been reported by Asboe-Hansen (3).

Slack (196) has studied the effect of limb atrophy on the loss of radioactive sulfate from various sulfate fractions obtained from such limbs. An increased rate of disappearance was observed from the fractions containing the mucopolysaccharides. Evidence was obtained suggesting that some portion of the $S^{35}$ was incorporated into polysaccharide fractions other than CSA which have substantially different turnover rates than does CSA. The decreased incorporation of $S^{35}$ in cartilage resulting from hypophysectomy can be partially restored to normal by growth hormones according to Ellis *et al.* (63). Daughaday *et al.* (46) have studied this phenomenon in greater detail and have shown that the accumulation of $S^{35}$ in cartilage varies directly with the log of the dose of growth hormone within certain narrow limits. This response has been proposed as an assay method for growth hormone.

The claim by Layton (123) that cortisone has a specific effect on inhibiting the esterification of sulfate in connective tissue has been questioned by Clark & Umbreit (35) on the basis of their finding that, although cortisone alcohol interfered with the incorporation of $S^{35}$ by cartilage slices, similar effects were not observed with hydrocortisone. Steroids with biological activities different from cortisone were inhibitory.

The enzymatic mechanisms involved in the esterification of sulfate are as yet unknown. Boyd & Neuman (29) investigated the uptake of radioactive sulfate by cartilage slices. The $Q_{O_2}$ of embryonic cartilage was found to be greater than that reported for adult cartilage. They noted that only part of the $S^{35}$ uptake was temperature dependent. Sulfate uptake was inhibited by anaerobiosis, methylene blue, malonate, and selenate, but stimulated by the presence of glucose. Dinitrophenol and molybdate were found to inhibit sulfate uptake without inhibiting respiration. The esterification of inorganic sulfate in liver has been studied by De Meio *et al.* (51) and Hilz (95) who found that this reaction involves ATP.

The suspected production of mucopolysaccharides by fibroblasts has been demonstrated in tissue culture by Grossfeld *et al.* (81). The material obtained gave a mucin clot which was inhibited by prior treatment with hyaluronidase. The rate of metabolism of mucopolysaccharides in rat and rabbit skin has been studied by Schiller *et al.* (185, 186). HA was found to turn over much more rapidly than does the CSA fraction which was demonstrated to consist of a mixture of two compounds, probably chondroitins A and B in the terminology of Meyer (155). Turnover rates were essentially similar when measured by turnover of the acetyl group, hexosamine, or the sulfate group of the CSA. This indicates that neither the $SO_4^=$ or acetyl group engage in exchange reactions. That sulfate of CSA does not exchange *in vivo* with inorganic sulfate has also been indicated by the experiments of Amprino (1).

Schiller & Dorfman (184) have reported that there is a decreased rate of synthesis of both HA and CSA in alloxan diabetic rats. Wick *et al.* (227) showed that insulin increases the intracellular transfer of glucosamine into

diaphragm. This transfer is associated with a substantial reduction in rate of transfer of glucose.

*Physical chemistry of acid mucopolysaccharides.*—The acid mucopolysaccharides are best interpreted as linear polyelectrolytes. The behavior of this class of substances, both synthetic and naturally occurring, has been discussed (70, 116). It has been shown for both CSA by Mathews (142) and for HA by Balazs & Laurent (7) that the electrical charge on the molecule and also the molecular configuration is a function of the concentration and type of small ions present in solution. This approach has been utilized in the interpretation of the action of inhibitors of hyaluronidase (144, 147).

A linear molecular structure for HA and CSA was indicated by streaming birefringence studies of Blix & Snellman (17). Molecular lengths for HA of different origins varied from 4800 Å to 10,000 Å and the corresponding molecular weights estimated to be in the order of 200,000 to 500,000. The preparations were molecularly polydisperse with a linear relation between intrinsic viscosity and average molecular length. Light scattering measurements on umbilical cord HA by Blumberg & Oster (18) indicated an M (weight average) of $8\times10^6$ and a shape equivalent to a highly hydrated spherical particle, 2100 Å in diameter. In a similar study Laurent & Gergely (122) reported an M of $2.8\times10^6$ to $4.3\times10^6$ and a shape approximated by a partially rigid coil with a root mean square radius of 2200 Å. It should be pointed out, however, that possible molecular polydispersity of HA, which affects particle shape determination, was neglected in both papers. An M value (152) of 23,300 by end group analysis is very likely too low. Molecular weights (number average) by osmotic pressure measurement vary from $3.16\times10^5$ to $1.75\times10^6$ (34, 107, 108). In these measurements, however, neglect of the Donnan effect may not be justified. Diffusion and sedimentation measurements at a single low concentration have been used to obtain an M of $5.2\times10^5$ (110). The partial specific volume of 0.86 reported is surprisingly high for a carbohydrate. A filamentous molecular structure for HA is deduced from electron microscopy by Jensen & Carlsen (109) [compare Gross (77)] and from irradiation data by Setlow & Doyle (190). Only diffuse diffraction patterns have been obtained upon x-ray examination of both HA and CSA (17). However, it is apparently possible to obtain a crystalline calcium salt of CSA (62).

Viscosity measurements have been utilized by many workers as a relative measure of the M of HA preparations. Most of the data are of little value, except for purposes of approximate comparison, due in part to the widely varying experimental conditions employed, and in part to neglect of hydrodynamic factors. The increase of viscosity due to HA in solution depends strongly upon concentration of HA, concentration and type of added electrolytes, pH, presence of trace protein, and heat treatment (167). In addition to these factors it is necessary to consider also, for highly viscous HA, the non-Newtonian character of the solutions and the temperature of measurement (207). A recent attempt to correlate visosity and M for HA preparations unfortunately neglects many of these considerations (108).

Flow potentials (111) and flow elasticity (112) in HA solutions have been

studied. Streaming dielectric data indicate strong interaction between HA and water (105, 106). No systematic investigaion of the electrophoretic behavior of HA or CSA has been made although much data may be found in papers mentioned here and in earlier references (75).

The composition and properties of the HA-protein complex of ox synovial fluid have been extensively studied by Ogston and co-workers and the conclusions summarized (162). The complex contains about 27 per cent protein and is a random coil of nearly spherical shape and very large hydration with an M of $1.2\times10^7$. It has been suggested that HA plays an important part in determining the lubricant properties of synovial fluid (163). Viscosity relationships have been extensively studied in pathological synovial fluids and correlated with various disease states (16, 68, 113, 164, 181, 207, 208). HA seems to play an essential part in the space network formation of vitreous gel (218).

Recent streaming birefringence and light scattering measurements (30a, 181a) show HA of vitreous humor with average M of $1.27\times10^6$ and a shape intermediate between a rod and a coil in approximate agreement with the results of Laurent & Gergely (122) for umbilical cord HA.

The M of "native" CSA of hyaline cartilage was estimated at 260,000 from streaming flow birefringence measurements by Blix & Snellman (17) and 43,000 from osmotic pressure measurements by Mathews & Dorfman (143). However, light scattering measurements on preparations freed of trace contaminants indicated an M of 50,000. CSA-protein complexes containing about 30 per cent protein were found to have molecular weights in excess of $1\times10^6$ (148). The physical properties of acid mucopolysaccharides of connective tissue other than HA and CSA have not been extensively studied. Some properties of a mucopolysaccharide-protein complex from cornea are reported by Woodin (232).

The physiological role of the acid mucopolysaccharides of ground substance has been a subject of considerable investigation and speculation. The regulation of capillary permeability by ground substance has been postulated by Duran-Reynals (57) and Gersh & Catchpole (74). The acid polysaccharides have been implicated in the binding and transfer of small ions and of water (121, 170, 181, 210), and in the stabilization of collagen (101) and elastic tissue (230).

The interaction of sodium, potassium, calcium, and other ions with connective tissue have been investigated by electrometric methods and the ion concentration changes correlated with colloid charge density and with alterations in glycoprotein "disaggregation" caused by hormones (66, 114). The binding of cations by cartilage is ascribed to CSA (28) which may play a specific role in the calcifying mechanism (6, 36, 65, 92, 133, 182, 200).

Vitamin A stimulates accumulation of bound sulfate in regions of active calcification (60). In vitamin-D-deficient rats there is an impaired utilization of CSA in the ossification process (61). The *in vitro* uptake of calcium by normal and pathological cartilage is related to the presence of CSA (13, 14). The acid mucopolysaccharides are associated with protein in the native state (153) and may stabilize collagen structure (101). Metachromasia of

CSA has been related to its polyelectrolyte properties (129). Sylvén (211) has suggested that metachromasia requires a certain minimum surface charge density and the presence of water and is due to a special type of orderly dye aggregation characterized by the formation of new intermolecular bonds between adjacent dye molecules.

*Hyaluronidase.*—The enzyme hyaluronidase continues to be the subject of a large number of investigations concerned primarily with the clinical use and conjecture regarding the biological importance of this enzyme. Space does not permit a review of these papers which have been enumerated with great thoroughness by Gibian (75).

Some progress has apparently been made in the purification of hyaluronidase. Malmgren (137) has reported certain physical chemical properties of several preparations of purified hyaluronidase including one obtained by Högberg, the method of preparation of which has not been published. This preparation was found to be electrophoretically homogeneous with mobility of $-3.4\times10^{-5}$ cm²/v/sec/at 0°. It was found to sediment as a single component with a sedimentation constant, $S_{20}^0$, of 1.2 S. Diffusion studies also indicated that the material was homogeneous. On the basis of ultracentrifugal and diffusion data, the M was estimated at approximately 11,000. Data are given for composition and amino acid content. This material behaved differently with respect to isoelectric point than did the activity of cruder preparations. It is suggested that native hyaluronidase may have a higher M, the enzyme being associated with some type of carrier *in vivo*. Some type of transformation is consistent with the heat stability in acid which has been reported (64, 145). Caputo (32) separated by paper electrophoresis an active component of crude hyaluronidase which was found to have an isoelectric point of 5.75. The absorption and purification of hyaluronidase on glass has been reported by Rasmussen (176). It is suggested that much of the reported instability of highly purified hyaluronidase may be due to absorption on the walls of glass containers.

The production of hyaluronidase by various bacterial species continues to attract some interest. Faber & Rosendal (67) found that, with the exception of types 9 and 28, at least one representative of each of 40 different Lancefield types of Group A streptococci produced hyaluronidase. HA formation was observed in at least one representative of 37 different types. Rogers (178) found evidence that hyaluronidase was a constitutive enzyme of *S. aureus* and that the lag in appearance of the enzyme was longer than the lag in growth.

Considerable interest still attaches to the phenomenon of inhibition of hyaluronidase. This subject has been reviewed in some detail by Mathews & Dorfman (147). Newman *et al.* (160) have isolated from human blood an apparently monodisperse substance which has the properties of the nonspecific inhibitor of hyaluronidase. The data obtained indicate that it is a labile protein containing carbohydrate (including hexosamine) with an M of approximately 100,000. No evidence for the presence of heparin was found.

Rogers & Spensley (179) have prepared synthetic polyanionic inhibitors of hyaluronidase by the treatment of equimolar amounts of hydroquinone

sulfonic acid and formaldehyde in alkali. These compounds, reported to have a low toxicity, were thought to be more active than those previously reported by Hahn and co-workers (84, 85, 86). A mixture of the polymer with semen resulted in inhibition of fertilization in the rabbit. Beiler *et al.* (12) pointed out that, whereas compounds which did not inhibit hyaluronidase *in vitro* were also inactive *in vivo* (as measured by effects on rate of absorption), some compounds active *in vitro* were not necessarily active *in vivo*.

The methods of assay of hyaluronidase have recently been critically reviewed by Tolksdorf (214) and Dorfman (54).

### Biological Aspects

Tinacci & Benassi (213) have studied the effect of intravenous administration of HA and CSA in rats. After the administration of 5 mg. per 100 gm. of body weight, 25.0 per cent of the HA and 31.5 per cent of the CSA could be recovered from the urine. Histological examination of organs of the injected animals showed a small increase in metachromasia, particularly in the kidneys, and formation of nodules in liver and spleen. Seifter *et al.* (189) studied the effect of the injection of 150 mg. per kg. per day of streptoccocal HA in mice, rats, rabbits, and dogs. No evidence of toxicity was detected, with the exception of slight evidence of thymic involution.

In an attempt to measure the relative amounts of fibers and ground substance in connective tissue, Sobel *et al.* (199, 201) have compared the concentration of collagen to that of hexosamine in bone and skin under a variety of conditions. It is difficult to interpret the meaning of the hexosamine determinations in view of the fact that hexosamine occurs in a number of proteins. The ratio of hexosamine to collagen in skin of rats was found to decrease with age. Rats receiving testosterone, $\alpha$-estradiol, and relaxin were found to have less than the expected amount of collagen, while those receiving parathormone and propylthiouracil had more than expected. Animals given $\alpha$-estradiol, salicylic acid, 12 per cent casein, relaxin, and cortisone showed significantly less hexosamine in skin than expected for normal animals. The ratio of hexosamine to collagen was found to decrease in rat femurs with increasing age. Boas & Foley (20) similarly found that the concentration of hexosamine in rat connective tissue decreases with age. They pointed out that the concentration of hexosamine in connective tissue is correlated with the water content.

Bárány & Scotchbrook (11) concluded that there is a hyaluronidase-sensitive barrier to the outflow of aqueous humor in normal eyes since, after treatment with testicular hyaluronidase, the resistance of the filtering angle of dead cattle eyes drops to about one-half of the original value.

The CSA content of human cartilage, as measured by hexosamine analysis, has been bound to be inversely proportional to age (133).

Hieronymi (94) claims that acid mucopolysaccharides occur in greater amounts in malignant than in benign tumors, and Benitz (15) has reported that increased amounts of mucopolysaccharides are found in experimental arteriosclerosis in the rabbit. In both cases conclusions were drawn on the basis of histochemical methods.

A number of reports have appeared concerned with the production of lathyrism in rats, a condition characterized by marked skeletal deformity produced by the feeding of the flowering sweet pea (*Lathyrus odoratus*). The active principle has been isolated and identified as glutamyl aminopropionitrile (44, 159, 187). Dasler (45) found that synthetic β-aminopropionitrile and β-mercaptoethylamine produced effects similar to the naturally occurring compound. Bachhuber *et al.* (5) reported similar results for synthetic β-aminopropionitrile. They found that methylation or acetylation of the amino group rendered the compound inactive. Ethylene cyanohydrin, trimethylene diamine, and β-nitroethanol had no activity. The effects of various diets on the production of lathyrism have been studied (4, 43). Dasler has suggested that experimental lathyrism may be the result of interference with metabolism of collagen (42).

A number of studies have appeared dealing with the effect of adrenocortical hormones on various aspects of connective tissue physiology. Sundblad *et al.* (209) have studied the effect of the intra-articular injection of hydrocortisone on the concentration and polymerization of synovial fluid HA, utilizing the intrinsic viscosity and anomalous viscosity as determined by their previously published methods (208). Clinical response to intra-articular treatment of rheumatoid arthritis with hydrocortisone was always reflected by an increase in concentration and increase in the degree of polymerization of the HA. They point out, however, that this may be secondary to the anti-inflammatory action of the hormone. Romani (180) showed that the administration of cortisone and hydrocortisone to rats with turpentine abscesses inhibits proliferation of fibroblasts and formation of new capillaries in the granuloma and increases the amount of mucopolysaccharide in the tissue surrounding the abscess. This confirms the previously published work of Taubenhous *et al.* (212). That the inhibition of wound healing by cortisone can be reversed by the local application of tissue extracts has been reported (158). Singer & Borman (193) have used the technique of Meyer *et al.* (157) consisting of measuring the granulation tissue which accumulates around implanted cotton pellets as an index of anti-inflammatory potency of a number of synthetic halogen substituted steroids. 9,α-fluoro-hydrocortisone acetate has been found to be 13 times as active as hydrocortisone acetate, and the corresponding chloro derivative has been found to be 2.8 times as active as the hydrocortisone acetate. Using a somewhat similar technique, DeSaulles *et al.* (52) found that aldosterone caused a slight increase in the formation of granulomatous tissue as a result of stimulation by a foreign body.

Ludwig (136) reports that the existence of scurvy does not prevent the production of experimental exophthalmous by thyrotropic hormone in the thyroidectomized guinea pig. Boas & Foley (21) have studied the effects of thyroxine and growth hormone on the hexosamine concentration and the amount of orbital connective tissue. It was concluded that the concentration and amount of hexosamine is determined by a balance between the thyroid hormone and growth hormone. Thiouracil was found by Harkness *et al.* (91) to cause an increase in total collagen of the thyroid gland. Williamson & Neumann (228) found that growth hormone in low doses in rats increased

the healing of experimental wounds, while in higher doses the opposite effect was observed; however, Spain & Molomut (202) found that growth hormone did not reverse the inhibition of wound healing produced by cortisone treatment in mice.

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# MUSCLE[1,2,3]

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## Introduction

In accepting the editors' invitation to prepare a review on "Muscle" the present reviewer agreed to limit the discussion to a few significant papers which would illustrate certain major trends or accomplishments in this field. Significance, like the gates of life (to paraphrase Ben Hecht),[5] is guarded only by chance and passion; hence the omission of certain important papers was accidental, while the inclusion of others was guided entirely by the reviewer's personal interest and prejudice. Since the problems discussed here represent certain phases of the molecular physiology of muscle on which definite advances were made in the past two or three years, there has been no attempt to isolate them in time, and a certain amount of earlier work has been reiterated for the sake of presenting the complete narrative.

*Books, reviews, and discussions.*—Szent-Györgyi's monograph (192), in the author's refreshing style, summarizes and integrates the views on the molecular physiology of muscle that have been developed by his school. Dubuisson's book (45) purports to delineate the most important work done on muscle during the first half of this century. A masterly account of the structural and physical chemistry of muscle proteins and of their purification and characterization is presented by Bailey (5) in Volume IIB of *The Proteins*. Among the valuable features of this chapter is the clear presentation of the mechanical and enzymatic properties of muscle models and of the significance of their behavior to the thermodynamics of muscular contraction. Valuable compendia upon the problems of ion transport (35, 230) and the diseases of muscle have appeared in book form (2).

Reviews of general problems in muscle physiology have been written by Mommaerts (138) and Gerard & Taylor (70); smooth muscle pharmacology has been reviewed by Furchgott (66) and Vaughan Williams (205), and

[1] The survey of the literature pertaining to this review was completed on August, 1 1955.

[2] The following abbreviations are used in this chapter: ATP (adenosinetriphosphate); ADP (adenosinediphosphate); EDTA (ethylenediamine tetra-acetate); AMP (adenosinemonophosphate); PC (phosphocreatine).

[3] A portion of the expenses incurred in preparing this review was defrayed by a contract between the Office of Naval Research and Stanford University, NR 102060.

[4] The reviewer's thanks are due to his good friend, Mr. Frederic M. Falconer, Head Librarian, School of Biological Sciences, Stanford, for providing the grist; to Mmes. Dorothy M. Maron and Mary S. Hurd for transcribing the reviewer's illegible script; and to Messrs. W. T., McElroy, Jr., and I. L. Trapani for arranging the bibliography and checking the citations.

[5] Hecht, B., *Count Bruga* (Boni and Liveright, New York, N. Y., 1926).

various aspects of heart muscle physiology have been treated by Gregg (75) and by Katz *et al.* (116). Polissar (150) and Ling (122) have presented stimulating theories of muscular contraction and energization, and Wilkie (221) has written a critical account of the recent theories of muscular contraction.

The present status of a number of specific problems in muscle physiology and biochemistry has been reviewed, as follows: transmission, by Fatt (54); denervation atrophy, by Fischer (61); contractile models, by Hasselbach & Weber (89), Weber & Portzehl (217), and Weber (213); innervation of skeletal muscle, by van Harreveld (84); contracture, by Sandow (172); structure by Bennett (15) and A. G. Szent-Györgyi (194); subcellular processes, by Harman (83); and certain aspects of molecular physiology of muscle by Hamoir (82), Mommaerts (137, 140), and Morales *et al.* (143a).

## Synthesis of the Work-Cycle

*Contractile analogs and models.*[6]—The application of the synthetic method to the solution of biological problems has been possible in only a few areas; it has been particularly fruitful in studies of muscular contraction. Thus the basic requirements for mechanochemical coupling, energy transfer, contraction, and relaxation have been reproduced in simplified systems by means of three general lines of approach: (*a*) the study of polyelectrolyte gels (115), (*b*) the investigation of extracted threads and fibers, and (*c*) experiments on glycerol-treated muscles.

A major success in muscle physiology, attributable almost entirely to the use of the synthetic method, has been the reconstruction of the working cycle and the establishment of the simplest requisites for its complete operation. Progress in this field has been very rapid, for as late as 1952 Weber & Portzehl (214) wrote, "For the relaxation phase, no model is yet known." In examining the reasons for this increase in theoretical orderliness one can point to several significant findings that were directly material. Among the most valuable contributions, historically, was the purification and physical characterization by the Szent-Györgyi school of the various proteins which form the basic contractile engine; instead of a mess, there became available a definite chemical entity, the actomyosin thread, which incorporated the minimum requirements for contraction. The early results were difficult to interpret because the threads prepared then did not have predictable behavior with respect to adenosinetriphosphate (ATP): they usually contracted (under insignificant or zero load) but frequently they extended. The early thread models were unoriented, and lacking the architectural reënforcement of lateral bonding, they developed little tension. Hayashi's (91) technique of spreading and compressing actomyosin monolayers in a Langmuir trough is the best of the current methods for producing oriented fibers. But even with the development of improved fiber models the significance of ATP in the

[6] Professor Bozler, to whom the author is indebted for critically reviewing the manuscript, has suggested that the term "reconstruction" rather than "model" should be applied to these simple systems.

contraction cycle remained in doubt until it was demonstrated that, in common with other pyrophosphates, ATP had a plasticizing (softening) effect which was unrelated to its role as an energy source. The softening effect of ATP was inferred, in a negative way, by Bate-Smith & Bendall (6, 7) who noted a parallelism between the onset of rigor mortis and the disappearance of ATP. Weber (215) showed that high concentrations of pyrophosphate relaxed glycerol-treated fibers which had been previously shortened by ATP and, recently, Bendall (13) re-emphasized that the configurations necessary to split actomyosin into its components are present in ATP, inorganic triphosphate, and pyrophosphate. Plasticization can be produced by any agent capable of interfering with secondary forces; thus the rapid relaxation brought about by urea was interpreted by Bozler (18, 24) to result from the splitting of hydrogen bonds. The fascinating story of the relaxation phase, culminating in the identification of the Marsh-Bendall (relaxing) factor as myokinase (12) was resolved primarily with the aid of the glycerol-extracted rabbit psoas. This preparation, introduced into muscle physiology by Szent-Györgyi (191), contains the contractile machinery *in situ* but is devoid of ATP, and does not spontaneously extend after contraction.

*Contraction-relaxation cycle in simple systems.*—Hayashi & Rosenbluth (90) found the conditions for shortening and relaxation to be the same in the glycerol-extracted psoas and the oriented fiber models. The requirements for shortening were the presence of ATP in low [KCl]; those for elongation were a high [KCl] with or without ATP. Since rapid elongation in the presence of a high (0.3*M*) [KCl] occurred only if the muscle were loaded, it was concluded that elongation of the actomyosin fiber was entirely passive. The stretch-release behavior of actomyosin fibers in ATP and 0.05 *M* KCl was studied by the same authors (92). Weber (212) obtained satisfactory agreement between the behavior of glycerol-extracted and normal muscles with respect to their reactions to ATP and to certain physical variables, and Portzehl (152) confirmed the similarity in the fundamental contractile processes existing in models and actomyosin threads exposed to ATP and inosine triphosphate. The effects of Ca and Mg on the interactions of F- and G-actin with myosin were studied by Spicer (184). Ulbrecht & Ulbrecht studied the rate of shortening and the efficiency of ATP-splitting on fiber models of *Anodonta* adductor (203) as well as on the glycerol-extracted rabbit psoas (204). The latter study showed that at 20°C ATP was present in the fiber core only if dephosphorylation was inhibited by the Marsh-Bendall factor. The power of extracted fibers was increased with temperature and, depending on the load, the ATP content was exhausted after a shortening of 25 to 40 per cent. ATP and uridyltriphosphate were compared by Ranney (155) with respect to their ability of inducing contraction in the extracted psoas and the ventricle of the dog, and of lowering the specific viscosities of purified actomyosin solutions prepared from these muscles. The two compounds were shown to have an equivalent effect (on a molar

basis) upon the contractile model, but, as judged from the viscometric results, uridyltriphosphate was less effective than ATP in its dissociation of actomyosin. Ranney (156) found the response of the glycerinated dog psoas to depend on the pH of the surrounding fluid phase, and he demonstrated that whereas the response to ATP in the presence of unbuffered KCl included a spontaneous relaxation, this phase was absent in buffered KCl, the muscle tending to maintain its maximum contractile force.

A proportionality between [ATP] and isometric shortening was found by Taeschler & Bing (195) for the dog heart and by Bowen (17), who concluded that the shortening observed in the extracted psoas did not directly result from ATP dephosphorylation. The contractile strength of the latter preparation was shown by Edman (48) to be maximally potentiated by $10^{-6}$M ouabain in the presence of $10^{-3}$M Ca, the glycoside being ineffective when tested alone. This finding recalls the prior demonstration of digitoxin effects upon extruded actomyosin threads by Mallov & Robb (127), and the subsequent results of Robb & Mallov (164), obtained with ouabain on Hayashi fibers. A variety of pharmacological agents has been found by Robb *et al.* (165) which shorten Hayashi fibers in the absence of ATP and by Waser & Volkart (208) that affect the viscosity of actomyosin solutions. In view of the fact that Wollenberger (224) reported that both the cardio-active and the cardio-inactive glycosides could polymerize actin, it appears premature to ascribe the inotropic effects of these agents to their behavior in simplified physical systems.

Any analysis of the relaxation phase must take into consideration the relevant data provided by studies of the interrelationships among inorganic cations, pyrophosphates, and the contractile model. The importance of ionic calcium in the working cycle of the model was stressed by Bozler (18) who found that the maintenance of tension in the presence of Ca and ATP proceeded under such circumstances as would provide a constant supply of energy; in the absence of Ca, ATP caused rapid relaxation. Relaxation was also produced by urea, pyrophosphate (18, 20) and Mg (25). Since this observation was not in accord with the evidence then available for the operation of the Marsh-Bendall factor, the problem was reinvestigated by Bendall (10) who showed that Mg was required for the pyrophosphate-induced extension of the ATP-treated preparation. He found that the Mg effect could be prevented by Ca and that, instead of lengthening, the fibers would shorten if the [Ca] exceeded the value necessary for inhibition. The influence of Ca and Mg on the mechanical (21) and enzymatic behavior of glycerinated fibers was examined by Bozler & Prince (22). Tension development and P liberation were found to be associated, generally, with the exposure of ATP-treated preparations to Ca or with the removal of Mg. Enhancement, rather than inhibition, was observed in aged or Ca-treated preparations when they were challenged with Mg, while relaxation and reduced enzymatic activity were accelerated by pyrophosphate.

Further investigations of the pyrophosphate-Mg problem (26) in-

dicated that the "softening" action of pyrophosphate could be prevented by previously treating the preparation with the metal-chelating compound, ethylenediamine tetra-acetate (EDTA). Plasticity could be restored by adding Mg, and the EDTA effect prevented if an excess of Mg were present when EDTA was added. Additional observations on the persistence of the pyrophosphate effect after washing with Mg, and of its prompt disappearance after bathing with KCl, led to the inference that pyrophsphate complexed with the protein and that the complex had a functional activity determined by Mg.[7] The inclusion of phosphocreatine (PC) among the test substances provided a better reconstruction of the working cycle (23); PC accelerated ATP-induced relaxation, reduced the [ATP] required for producing relaxation or contraction with Ca, and, in the presence of adenosinemonophosphate (AMP) gave a slow S-shaped contraction curve from which the author inferred that AMP was being autocatalytically converted to ATP. Phosphocreatine could produce contractions in a preparation washed free of ATP if the elapsed time between the two operations was less than five minutes. Acceleration of both phases of the working cycle was attributed to the increase in transphosphorylation owing to the presence of PC in the system.

*The relaxation factors.*—Since the successful elaboration of this problem represents a major achievement in recent efforts to reconstruct the working cycle of muscle, a somewhat detailed account will be given of the experiments leading to the purification of the relaxation factors and their identification as enzymes.

The existence in muscle extracts of a water-soluble, nondialyzable factor that inhibited adenosinetriphosphatase activity and prevented the syneresis of muscle homogenates was reported in 1951 by Marsh (128). He found that the volume reduction normally exhibited by a washed brei after adding ATP could be prevented if the system were first treated with KCl extracts of fresh rabbit muscle. Subsequent work (129) showed a correlation between the presence of the factor and the reduction of phosphate splitting. The factor could be inactivated by prolonged dialysis against distilled water or by heat, and was judged to be an acid-labile protein that was different in its effects from a number of relatively pure glycolytic enzymes (including creatine phosphokinase) which were tested. Syneresis was promoted by Ca. Reversible swelling occurred when fibers were tested with ATP in the presence of the factor but only irreversible shrinking was observed in its absence. The characteristic activity of the factor was not inhibited by iodoacetate and this was confirmed by direct microscopic observations on fiber particles. Bendall (9) showed that a stable extract could be obtained [from the muscles of Myanesin-treated rabbits (8)] if it were prepared and dialyzed in the pH range 6.8–7.1. Tests on iodoacetate-poisoned and glycerol-treated fibers indicated that in both cases the ATP contraction could be reversed by the

[7] Recent chemical experiments made by Bozler (226) indicate that washed fibers contain tightly bound Mg which can partly be removed by pyrophosphate.

addition of the relaxing factor, and that the glycerol-treated preparation so lengthened would recontract if Ca were added. An extensive study (11) of the ionic requirements for relaxation of ATP-shortened fiber models indicated that the factor would operate only in the presence of Mg. Conversely, experiments on well-washed and poorly-washed preparations showed that, in the former case, the apparent relaxing effect of Mg was attributable to traces of factor still adsorbed on the fibers. Shortening, induced by KCl-ATP solutions, was enhanced by Mg if the factor were absent, but under these circumstances Ca was ineffective. Incompletely washed fibers, or fibers treated with dialyzed "factor," showed relaxation with Mg and contraction with Ca [cf. Bozler (19)] and in all cases the adenosinetriphosphatase activity was affected in the same way as contraction. Since Ca, in the absence of factor, was shown to have no shortening properties of its own, Bendall confirmed the finding of Bozler (19) that Ca has no direct function, but acts as an inhibitor of the intrinsic factor. The factor could be rendered inactive, even in the presence of high [Mg], by a slight reduction of the total ionic strength. Electrophoresis showed the active material to migrate with the myogen fraction.

An aqueous extract of freshly minced muscle was reported by Goodall & A. G. Szent-Györgyi (72) to contain a material capable of relaxing (and reducing the elastic modulus of) the ATP-treated glycerated fiber model. This effect was strictly pH-dependent, relaxation occurring in the range 6.2 to 6.6 and contraction at higher values. A chemical investigation of the active material, using the pH dependence of relaxation as an assay method, resulted in the isolation of a crystalline material which was identified as phosphocreatine. Maximal relaxation, in the pH range 6.2 to 6.6., required 10 *mM* PC and 2 meq. Mg, the effect being inhibited by 0.8 meq. Ca. The relaxing effect of PC was not demonstrable when tests were conducted on fibers that had been aged in glycerol-water or had been extracted with 0.05 *M* $NaHCO_3$-glycerol, but could be restored by incubating the models with dialyzed muscle extract. The active relaxing factor appeared in the fraction precipitating in 50 to 70 per cent saturated ammonium sulfate, and was found to be resistant to extensive dialysis. The impairment of the contraction phase that also resulted from prolonged bicarbonate-glycerol washing could be reversed by adding cysteine or acid-boiled muscle extract.

On the assumption that relaxation required the reconstitution of ATP, Lorand (123) surmised that the Goodall-Szent-Györgyi factor was an enzyme, probable adenosinetriphosphate-phosphocreatine transphosphorylase which might act by catalyzing the reaction $PC+ADP \rightleftharpoons C+ATP$. Proceeding on this basis, Lorand isolated the enzyme and showed during his purification process that the enzymatic and relaxing properties were both concentrated 18 to 30 times with respect to the original extract. An electrophoretically homogeneous preparation of ATP-PC transphosphorylase was found to be a highly potent relaxing agent under the conditions of the Goodall-Szent-Györgyi assay. Since both the relaxing and enzymic actions in the

presence of PC required a low pH and were inhibited when it was raised, Lorand suggested that the alternation of contraction and relaxation might be initiated *in vivo* by similar pH changes. Because relaxation appeared to be associated with rephosphorylation, Lorand pointed out that other enzymes, e.g., myokinase, which can produce ATP from ADP, might also be effective as relaxing agents, but that they would be less important than transphosphorylase because their action was slow. He suggested that the Marsh-Bendall factor might be either of the above, but implied that it was probably transphosphorylase.

Bendall (14) established his preparation to be myokinase and showed that it differed from Lorand's product in two important respects. Whereas ATP-PC transphosphorylase displayed its maximal effect at pH 6.2 and was inactive at pH 7, myokinase provided optimal relaxation around neutrality (pH 6.8 to 7.2) but was inhibited in the lower range (pH 6.0 to 6.5). The second basis of difference was the necessity of PC for the operation of the Lorand cycle but not for myokinase, and Bendall stressed that the presence of PC was precluded by the way in which his factor was prepared and used.

An extensive series of investigations (12) disclosed the relaxing effect and the myokinase activity to be highly correlated in myokinase preparations of varying degrees of purity. Electrophoretic analyses of the original extract, crude myokinase, and of two preparations purified by trichloroacetic acid-ammonium sulfate treatment showed four components, the activity being restricted to a common electrophoretic individual, *b*, which could not be obtained entirely free of a small amount of an inactive component, *c*.

In the presence of ATP and Mg, myokinase depressed the adenosinetriphophatase behavior of glycerol-extracted fibers and promoted lengthening; but these effects could be reversed by adding small amounts of Ca. On the hypothesis (87) that the production of pyrophosphate was the underlying cause of myokinase-induced relaxation, Bendall (12) compared the enzyme-inhibiting properties of the several preparations with those of pyrophosphate solutions. In the presence of Mg the adenosinetriphosphatase activity of the variously treated fiber preparations was reduced by 64 to 76 per cent, the maximal reduction being due to 4 $mM$ pyrophosphate, and in all instances enzyme inhibition could be reversed by adding Ca. Inhibition was not observed if the [Mg] was reduced from 4 to 0.4 $mM$. If the ionic strength of the medium were reduced from $\mu = 0.18$ to $\mu = <0.04$, myokinase would neither inhibit enzymatic activity nor would it promote relaxation; in this respect it differed from pyrophosphate, which was much less dependent on the ionic strength [cf. Hasselbach (88)]. Adenosinetriphosphatase activity of the fibers was not affected by AMP or by inosine monophosphate in the presence of myokinase but it was slightly increased by ADP. None of these compounds influenced the lengthening behavior.

(*a*) Mechanism of relaxation: A strong link in our understanding of the interdependence between contraction and relaxation has been provided by the identification of the relaxing factors, and it is apparent that detailed

knowledge of the physical chemistry of their interaction with the contractile apparatus is needed before the working cycle can be successfully reconstructed. Since inhibition of adenosinetriphosphatase, rather than classical myokinase activity, appears to be central to the relaxing effect of the Marsh-Bendall factor, it is relevant to examine its behavior with respect to Ca and Mg, and to understand the mechanism whereby such compounds are bonded to participating groups on the actomyosin molecule.

The likelihood that the relaxing factor might operate by masking sulfhydryl groups, known to be involved in the enzymatic breakdown of ATP, was a natural consequence of the demonstration that organomercurial inhibitors such as mersalyl (Salyrgan) prevent both the enzymatic and contractile functions of models. This view has been proved untenable by Portzehl (151) who showed that mersalyl inhibited only the enzymatic behavior of actomyosin but not its response to the plasticizing action of ATP. That the binding of ATP in relaxation and contraction does not involve the same groups on actomyosin was further exhibited by the fact that the ATP was not split during the relaxation phase; thus disinhibiting the blocked adenosinetriphosphatase groups with cysteine restored both the enzymatic and contractile properties of actomyosin.

However, as Weber & Portzehl (217) have pointed out, deductions based on mersalyl experiments are not unequivocal, and the fact that adding mersalyl and ATP at almost the same time results in relaxation can be explained by assuming that inhibition of adenosinetriphosphatase is a faster process than the poisoning of plasticization. The problem of mersalyl has received extensive treatment by Weber & Portzehl (214) and Bailey (5). The fact that the relaxing factor does not react with the same groups as mersalyl because of its dissimilarity with respect to ATP-dependence (86), ionic strength requirements (12), and cysteine reversal (88) has been emphasized.

Previously cited evidence obtained on models (129), has indicated that the Marsh-Bendall factor is not inhibited by iodoacetate. It is significant to note, both in this connection and with respect to its bearing on work-cycle hypotheses, that Sandow & Mauriello (171) greatly increased the relaxation rate of iodoacetate-poisoned frog sartorii by treating them with inorganic pyrophosphate. In an extensive discussion of the mechanism of iodoacetate rigor Sandow & Schneyer (173) pointed out that there is yet no evidence to suggest the participation of the Marsh-Bendall factor in the subsequent relaxation of iodoacetate-poisoned muscles; however, from the fact that relaxation is dependent on the plasticizing property of ATP, it could be construed that the lengthening reaction was being brought about by the pyrophosphate derived from the small amount of intact nucleotide remaining in the muscle. Additional information on work-cycle inhibitors is provided by Hasselbach (88).

The close analogy between the effects of EDTA and the Marsh-Bendall factor on the behavior of glycerol-extracted models has yielded a convenient

system for studying the functional interrelationships of the alkaline-earth cations in the enzymatic and nonenzymatic phases of the cycle. The role of Mg in contraction and relaxation and the method whereby certain of these effects are influenced by Ca were clarified by Bozler's (27) experiments performed with the aid of EDTA. On the basis that EDTA closely imitated all the effects of the natural relaxing factor, he was able to conclude that relaxation was caused by the inactivation of bound Ca and was accompanied by the formation of an enzymatically inactive ATP-protein complex. Qualitatively similar but less extensive studies were made by Watanabe (211) on the relaxing behavior of EDTA. His experiments on the pH dependence of relaxation suggest certain operational similarities in the actions of EDTA and the Lorand factor. Further information concerning the influence of EDTA on enzymatic activity is provided by Friess (63) and Friess *et al.* (64).

(*b*) Theories of action of the Marsh-Bendall factor: Now that the relaxing substances, particularly the Marsh-Bendall factor, have been shown to be enzymes the interesting problem that arises is this: do these substances exert their effects by acting as enzymes, or do they bring about relaxation by removing certain materials necessary for activating contraction? It is patent that the answer to this question will exert a significant influence upon the fate of the recovery-energization hypothesis. In view of this, it must be pointed out that the relationship between the biochemical and the mechanochemical aspects of myosin-adenosinetriphosphatase behavior is by no means certain (53, 62, 74, 139, 141).

The views of the Weber group (216, 217) and Bozler (27), regarding the operation of the relaxing factor, are essentially alike in that they both consider inhibition of adenosinetriphosphatase activity to be its central function, and in this respect they differ from Bendall's hypothesis (12) that relaxation is not brought on directly by the plasticizing action of ATP, but through the production of pyrophosphate by the activated myokinase.

The preëminent role of the alkaline earth metals as enzyme activators and ligands is considered in all of the schematic cycles [cf. Sandow (168)]; Weber & Portzehl (217) attach particular significance to the strategic displacement of these metals in the fibril in connection with the transfer of excitation energy. According to the latter, if a disturbance, started by a shift in electric field, could move Mg a distance of some 100 Å, inactivating the relaxing factor and simultaneously activating the actomyosin, contraction would be initiated. The effect would be further strengthened if the Ca were attached to the relaxing factor during the phase of contraction and to actomyosin during that of relaxation. Bozler's scheme (27), based on his EDTA experiments, is somewhat similar. He considers three conditions: rigor, relaxation, and contraction. In rigor the relaxing factor is attached to actomyosin through Ca; combination with ATP and Mg transforms the muscle to a plastic condition and produces an enzymatically inactive complex. If Ca is then added, it apparently combines with the factor and, by removing it, unmasks the protein-bound Ca. This renders the contractile protein enzymat-

ically active, and contraction ensues. It is significant to note that in this scheme contraction is initiated by the bound Ca, the well-known inotropic effect of the free ion being to inactivate the relaxing factor.

Bendall's reconstruction is in certain respects somewhat more detailed. He disposes of the possibility that the relaxing cycle represents the inhibition of classical myokinase activity for under those circumstances it could be expected that adenosinetriphosphatase inhibition might be produced by the accumulation of reaction products, none of which has been shown to induce lengthening. Instead, he suggests that myokinase might form an adsorption complex with the contractile structures that could operate by directly blocking contractile sites, or by catalyzing the breakdown of ATP to form pyrophosphate. The possibility of the latter reaction was predicated on the apparent similarities that existed between the effects of pyrophosphate and those of "factor-myokinase" on adenosinetriphosphatase behavior and shortening. Thus in the presence of Ca and Mg, "classical" myokinase activity would predominate, and contraction ensue; in the absence of Ca, the "new" reaction would be favored, in which (according to the author's diagram) one of the intermediate products is an actomyosin-pyrophosphate compound. The relaxed state is viewed, apparently, as the dissociation of actomyosin into its components under the influence of pyrophosphate. In support of his belief that the ATP molecule might be coupled through the phosphate group and the myokinase through the amino group, the author cites the fact that adenosinetriphosphatase can hydrolyze polyphosphates having no amino group, while the action of myokinase is restricted to adenine nucleotides.

Since the reviewer's purpose in the preceding section has been to present a detailed account of the work leading up to the present status of the relaxation cycle, the description had necessarily to be limited to a few representative papers, and many significant reports were not mentioned. Some of these are included in the bibliography (105, 112, 118, 119, 154, 202).

## Structure and Chemical Composition

*Denervation atrophy.*—The changes occurring in the protein content and enzymatic activity in denervation-atrophy were the subject of a review by Fischer (61). Evidence was marshalled to show that atrophy was accompanied by an increase in collagen and by an exponential decrease in muscle wet weight and muscle-phase volume. Changes in the important metabolites and ions were considered. A slight drop in the noncollagenous fraction of the muscle-phase was accompanied by an increase in myoalbumin, and by reductions in myogen, actin, and myosin. The author's recalculations of other workers' results showed enzymatic changes in the muscle-phase during denervation atrophy to include a sharp but unsustained rise in the activities of hexokinase, phosphorylases (II & IV), and in the content of glutathione. An eventual decline in cytochrome oxidase, dehydrogenase (after a brief increase), and lactic acid formation was noted. Recent papers not covered in that review include (59, 60, 77, 111, 175, 178, 179, 186, 196).

*Histology, histochemistry and ultrastructure.*—Aloisi *et al.* (3) showed a reduction in the positive birefringence of muscle and a loss of its striation to accompany the extraction of actomyosin with alkaline solvents; the intense negative birefringence was ascribed to the presence of depolymerized actin. Philpott & Szent-Györgyi's electronmicrographic studies (148) showed protofibrils to be composed of alternating layers of contractile and elastic material, the latter being located in the middle of the sarcomere. It was suggested that the elastic material could be tropomyosin, which has no contractile property. The significance of these findings to the identification of the "series-elastic element" was considered. The development of a new microtome by Hodge, Huxley & Spiro (103) permitted them to prepare sections about 300 Å thick. The results of studies made with these ultrathin preparations have been reported by Huxley (107) and Hodge *et al.* (104). The absence of cross-striations in the wing muscles of the bumble-bee was reported by Philpott (149).

Structural changes occurring during the contraction of living muscle were studied with the phase-contrast microscope by Huxley & Niedergerke (108, 109). They found that the dimensions of the A-bands remained constant under a variety of conditions and that lengthening or shortening of the sarcomere was associated with similar changes in the I-bands. These structural changes were assumed to depend on fiber-length and were judged to be independent of tension development. It was proposed that the dimensional changes in the I-bands represented the extension and shortening of actin filaments and that, during contraction, for instance, these could be drawn into the A-bands between the myosin rodlets. Effectively similar conclusions were reached by Huxley & Hanson (110), who studied the behavior of stretched and contracted myofibril ghosts that remained after myosin had been extracted with pyrophosphate and $MgCl_2$. Carlsen & Knappeis (33) found significant differences to exist between the lengths of striations observed in fixed and in living fibers; they argued that the A-band shrivels on fixation and, hence, that stretch is not reflected to the same degree in the extension of A- and I-bands. Studies in living muscle showed the A-bands to extend under stretch. Certain aspects of the cross-striation problem were investigated by Guba *et al.* (76) and Ernst (51).

A study of fragmented and sectioned cardiac muscle was made by Weinstein (218) with the aid of the electronmicroscope. Extensive branching and anastomosis of myofibrils was found. Myofilaments prepared from the sectioned material showed structures at intervals of about 220 Å that formed threads connecting with other myofilaments. Additional studies of cardiac histology included those made by Robb (163) on conducting tissue; by Cleland & Slater (36, 37) on sarcosomes; by Menschik (133) on elastic tissue; and by Dumont & Drouin (46) upon the selective occurrence of acetylcholinesterase in such regions as the sinus node, node of Tawara, and the bundle of His. The development and distribution of adult myosin in embryonic chick hearts was studied immunologically by Ebert (47).

*Physical biochemistry of muscle proteins.*—The development of an improved method permitted Tsao & Bailey (197) to prepare electrophoretically

pure actin by extracting dried muscle residues with acetone solutions containing ATP and ascorbate. Since only actin was selectively removed by this method, it was noted that the major muscle proteins could be obtained in electrophoretically homogeneous form from the same muscle mince. It was suggested that, *in situ*, actin tends to complex with the stroma through liponucleoprotein linkages. A study of the influence of various solutes on the osmotic pressure, viscosity, and fluorescence polarization of actin (198) showed that its fundamental unit was a monomer having a molecular weight of 70,000 which could dimerize by end-to-end association, probably via nucleotide prosthetic groups and an alkaline-earth cation.

In connection with their osmotic pressure data it is interesting to note that, although all of the samples tested were electrophoretically homogeneous, the relationship between the reduced osmotic pressure (P/C) and concentration (C) was variable, except at infinite dilution, and in the presence of ATP the dilution effect was much less prominent. While one preparation had a positive slope with respect to concentration, the remainder had negative slopes in the region below 1 per cent protein, and zero slopes above that limit. The reduced osmotic pressure values used to construct one of the functions were much lower than those in other experiments and suggested a number-average particle weight of about 140,000 in the concentration range (1 to 2 per cent) studied. The mean molecular weight of the monomer was found to be 70,000 by fluoresence-polarization and 74,000 by osmotic pressure. The dimeric form was taken to represent the kinetic unit of actin. ATP appeared to reduce the viscosity of depolymerized actin solutions.

The interaction of myosin, actin, and ATP [Tsao (199)] was studied by means of changes in the fluorescence-polarization of dye-coupled actin (1-dimethyl-aminonaphthalene-5-sulfonyl chloride). The results suggested that F-actin did not form polymeric fibrils in solution and that it dissociated into monomers during its reaction with myosin. Dimerization resulted upon adding ATP. On account of its theoretical significance the distinction between the effect of ATP on actin alone and upon actin in the presence of myosin should be noted: in the first instance, monomer formation was favored while, in the second case, recombination of monomers into dimers occurred.

Physical measurements made by Tsao (200) upon fractions of depolymerized myosin showed it to be composed of two types of subunits, viz., 92 per cent of highly asymmetric particles ($M_n = 165{,}000$) and 8 per cent of smaller fragments ($M_n = 16{,}000$), with axial ratios of 30 Å for the former and 12 Å for the latter. There appeared to be no evidence that tropomyosin was a constituent of the myosin molecule, the small amount of contaminant in "once-crystallized" myosin being removed on further recrystallization. Evidence for the separate existence of adenosinetriphosphatase was negative; the residual enzymatic activity that remained after myosin "denaturation" was attributed to traces of undenatured myosin. An extensive discussion of the interactions of actin, myosin, and tropomyosin was given by Tsao & Bailey (201). Tropomyosin molecules in solution were interpreted by Wasserman to behave like flexible chains (210).

Concurrently, additional evidence for the existence of functional subunits of myosin was established by the careful work of Mihalyi & A. G. Szent-Györgyi upon the physical and enzymatic properties of fragments obtained after digesting the parent molecule with trypsin. The authors observed in preliminary experiments (134) that, during the first phase of trypsin digestion, myosin was split without residue into heavy particles of two different sizes which could then be slowly despeciated if the enzymatic treatment were prolonged. The mean intrinsic viscosity of the combined components was 0.56, as contrasted with a value of 2.05 for the starting material, and characterization by ultracentrifugal analysis revealed that the fast and slow components had sedimentation constants of 5.3 and 2.5, respectively. Parallel studies on the actomyosin complex showed it to be digested at the same rate as myosin but that during digestion only the slow component of myosin was released, the fast one being retained in the digested complex. A detailed study of digestion kinetics characterizing the two phases, made by Mihalyi (135), indicated that the process underlying the first phase of digestion was first-order, and had a maximum rate at pH 7.8. The following thermodynamic properties were calculated from the dependence of reaction rate upon temperature: $\Delta H = 30{,}700$ cal., $\Delta F = 18{,}900$ cal., and $\Delta S = 39.8$ entropy units.

The fact that both myosin and actomyosin appeared to be attacked at the same rate during the first phase of digestion while the digestion of actin was much slower, led to the inference that the viscosity drop observed in trypsin-treated actomyosin resulted entirely from the digestion of the myosin component. On the basis that combination with actin did not prevent its being digested by trypsin, nor that treatment with trypsin affected its affinity for actin, it was judged that the two reactions probably operated through different sites on the myosin molecule, these sites being so greatly separated as to preclude any mutual influence. In addition to having their actin-binding properties unaffected by enzyme treatment, trypsin digestion did not effectively alter the enzymatic behavior of myosin molecules. According to Mihalyi & A. G. Szent-Györgyi (136) the adenosinetriphosphatase effect seemed to be concentrated in the faster moiety. The isolation and characterization of these subunits (meromyosins) was due to the further work of A. G. Szent-Györgyi (193). The two meromyosins [L (light) and H (heavy)] were separated according to their solubilities in KCl-ammonium sulfate solutions, and the L-meromyosin was crystallized. Physical measurements performed on the two subunits gave the following molecular dimensions for L- and H-meromyosin, respectively: molecular weight, 96,000 and 232,000; length, 550 and 435 Å; width, 16 and 29 Å. H-meromyosin was shown to be responsible for all of the actin-binding behavior and adenosinetriphosphatase activity of the myosin molecule, while L-meromyosin was considered to determine the solubility properties of the parent molecule.

The results of speculations based upon calculated dimensions of the meromyosins led the author to propose that one myosin molecule consists of two H- and four L-meromyosins linked in series, the latter forming the contractile

unit, and the former acting as an enzyme and as a junctional element for linking actin. Further investigations of the structural properties of L-meromyosin were made by Philpott & Szent-Györgyi (148), and of their physical-chemical behavior by Lauffer & A. G. Szent-Györgyi (120) and Nanninga (144, 145). A new fibrous protein, unrelated to actin, has been described by Amberson *et al.* (4).

## Biophysics of Resting and Active Muscle

*Mechanical properties and physical behavior.*—The destruction of beautiful theories by ugly facts is an acknowledged truism, but for that it is no less applicable. In the construction of theories the muscle physiologist is kept in bounds by two major considerations: that of thermodynamics which, in one respect, tells him what the system cannot possibly do; and that of structure, which gives him a basis for interpreting what the muscle does under a variety of circumstances. The past history of muscle physiology contains many notable examples of the rejection of theories on thermodynamic grounds but until recently, owing to the absence of precise knowledge regarding the arrangement and physical behavior of the various structural elements, it has not been possible to challenge them on the basis of engineering. It will always remain as a tribute to A. V. Hill that a good deal of the energetics of muscular contraction can be handily attributed to the inherent kinematic properties of the muscle substance. Thus, Hill (93), by relating the experimentally observed results that the rate of extra energy liberation was a linear function of the developed tension between the isometric tension, $P_0$, and the load $P$,

$$b(P_0 - P) \qquad 1.$$

(where $b$ = increase of energy rate/g.wgt. decrease in load; $P_0$ = maximum isometric tension; $P$ = developed tension) and the fact that the extra energy must be composed of the heat liberated on shortening ($ax$) and the work done ($Px$), giving $(a+P)x$ as the total energy and

$$(a + P)dx/dt \quad \text{or} \quad (a + P)v \text{ as its rate,} \qquad 2.$$

obtained the identity that

$$v(a + P) = b(P_0 - P); \qquad 3.$$

whence, by rearrangement, he got

$$(P + a)(v + b) = (P_0 + a)b = \text{constant,} \qquad 4.$$

the so-called "characteristic" equation of muscle. The force-velocity curve so produced is a rectangular hyperbola that fits well with experimental results, and the equation permits one to obtain the values of $a$ or $b$ from both force-velocity and heat-length data. This apparently fundamental description of muscle behavior obtained by Hill has withstood the operational tests to which he and others have subjected it over an extended period. Some recent applications have been made by Abbot & Wilkie (1), who showed that, by a simple modification, the equation could become valid over the full extent of

shortening, and by Macpherson (125) who observed that both the force-velocity curve of the contractile component and the relation between force and extension of the series-elastic element could be derived from a single pair of isometric contractions. Ritchie (160) obtained force-velocity curves for strips of rat diaphragm at 37°C. that were hyperbolic and in certain respects similar to those obtained on frog muscle. The average value of the characteristic relationship $a/P_0$ was 0.25 [cf. Hill (93)]. Human calf-muscles, excited by constant submaximal stimuli, gave force-velocity curves that agreed with Hill's equation. Bigland & Lippold (16) found that, at constant shortening velocity, the electrical activity was directly proportional to the tension and concluded that tension, velocity, and electrical activity were interdependent, the integrated electrical record providing a measure of the number of active fibers. Anionic modification of the Ringer solution was shown by Sandow & Mauriello (170) to affect the constants of Hill's equation.

Polissar (150) sought to provide a rigorous treatment of the phenomena of muscular contraction based upon the physico-chemical behavior of high polymers. Some of the fundamental assumptions involved are: (*a*) the contractile material consists of minute, serially-arranged elements composing a filament; (*b*) they have two states, *L* and *S*, and, by reacting with their environment, the elements can be transformed from one condition to another by means of separate reactions involving different intermediates, the length of the contractile material being the result of an equilibrium determined by the proportion of fibers in the *L* or *S* states. Diagramatically, the cycle

goes to the right by reaction I and to the left by reaction II, each process being characterized by first-order kinetics. Since the rate-constant is dependent on the temperature and on the activation energy, Polissar assumes that the way a stretch influences the reaction is to change its activation energy; thus, applying tension to the muscle would reduce $L \rightarrow S$ but to increase $S \rightarrow L$. In certain respects Polissar's treatment of the force-velocity relationship provides a better basis for predicting the results than Hill's formulation, particularly at increased loads where the muscle is known to extend. The status of the Hill equation was recently considered by Rosenfalck & Buchthal (167) in the light of the "transmutation" theory, proposed earlier by Buchthal, Kaiser & Rosenfalck (28), that contains the same fundamental elements as Polissar's.

Recent work on the mechanical properties of muscle has included studies of the torsional elasticity of isolated skeletal muscle fibers by Sten-Knudsen (185), and of stress-relaxation of smooth muscle by Zatzman *et al.* (225). Wilkie (220) and Ernst *et al.* (52) reported on the coefficient of thermal ex-

pansion of muscle and Mazzella & Bauer (132) observed pressures of about 200 mm. Hg to be developed within contracting muscles. Apparatus for measuring muscle strength was developed by Darcus (44) and by Walters & Harris (207). The relative contributions made by the contractile and series-elastic elements to the development of tension were studied by Hill (95) upon the isometrically contracting frog sartorius. The tension fell to zero when the muscle was allowed to shorten, but was then redeveloped at the shorter length. He interpreted these results as being indicative of the release of the series-elastic element in the first phase and the shortening of the contractile element in the second. The rapid increase in temperature accompanying the first phase was ascribed to the fall in tension and was not considered to result from physiological shortening (96). The similarity of contracting muscle in respect to its thermal behavior to that of materials having a normal coefficient of linear expansion was noted, as well as its difference from bodies having a long-range elasticity. Resting muscle, on the other hand, was shown to display long-range elastic behavior. It was argued that the force developed by a contracting muscle is not of thermokinetic origin and that the tension is derived from internal energy, not entropy [cf. Morales & Botts (143)]. The relaxation heat was considered to be provided partly from the elastic energy stored in the series-elastic component, the rest being due to the thermoelastic heat obtained in consequence of the fall in tension. An increase in tension was observed by Walker (206) to succeed a sudden stretch applied to the tetanized triceps surae, contracting isometrically at lengths other than that required to show maximal tetanic tension. For a given length, the extensibility was shown to be smaller in the tetanized than in the resting muscle and it was indicated that the excess tension produced could be attributed largely to passive resistance to extension. The $Q_{10}$ of the maximum tetanic tension developed by isolated frog muscle fibers was found to vary between 0.96 and 1.10 in a series of eight experiments reported by Washington *et al.* (209).

Apart from the classical length-tension relationship a variety of effects has been observed to accompany stretch or some other deformation of the muscle. Harris (85) observed a doubling of the rate of sodium extrusion in stretched frog sartorii, the effect disappearing during the summer. The implications of the stretch-induced increase in metabolic rate in activating the sodium pump mechanism were considered by the author. The oxygen uptake of muscles in which the excitatory mechanism had been poisoned with the alkaloid ryanodine was found to depend on their length [Edwards & Flinker (49)]. Bülbring (29) found that mechanically or pharmacologically produced increments in tension would raise the oxygen consumption of the isometrically contracting taenia coli. The mechanism of pressure-induced contraction was considered by Hamann (81) to involve a pressure-dependent dephosphorylation step. Ralston & Libet (153) found that stretching increased the amplitude of the action potentials of frog muscles when they were stimulated by maximal nerve volleys but not when stimulated directly. They considered this effect to result from the greater number of participating fibers,

stretch inducing the usually nonresponding fibers to react. X-irradiation (30 to 150 kr) produced an immediate impairment in muscle function and in accompanying changes in its length and appearance [Gerstner *et al.* (71)].

*Static-dynamic relationships of smooth and cardiac muscles.*—It is the intent of this section to consider by means of appropriate examples the fundamental behavior of smooth and of cardiac muscle. Pharmacological aspects will be treated only to the extent that they aid in illustrating some biophysical problem or some physiological property of the tissue.

(*a*). Smooth muscle: The effects of temperature upon the time-course of active and passive extension were studied by Kitisin & Gilson (117) on strips of longitudinal muscle prepared from rabbit stomach. It was concluded that normal relaxation and load-induced lengthening were mediated by separate processes, the former not involving the visco-elastic properties of the muscle. Singh & Sharma (181) found that preparations of frog stomach lengthened but that the sartorii shortened when exposed to $BaCl_2$ and NaCN, and that smooth muscle of *Mytilus* lengthened under the same circumstances. Reasoning by analogy, Singh & Singh (182, 183) inferred that relaxation involves a process akin to protein denaturation. The influence of temperature and stimulus strength upon the isometric and isotonic contractility of the uterus was studied by Csapo (41), and further investigations of its shortening kinetics in terms of the usual parameters were made by Csapo & Goodall (43) in relation to the action of sex hormones. Since these investigations appear to represent one of the most comprehensive attacks upon the dynamics of smooth muscle, their presentation in fair detail seems warranted.

The stimulus-response characteristics of smooth muscles being by tradition ill-defined and unreliable, it is evident from the account given by this group that the success of their studies was owed chiefly to the development of an adequate method of stimulation, and, secondly, to their clearly distinguishing in their experimental approach between isometric and the isotonic aspects of uterine behavior. Optimal response was secured by applying an A.C. stimulus for 5 sec. Varying the strength of stimulation produced a progressive increase in the amount of isometric tension but did not effectively alter the extent of isotonic shortening. A further difference between the isometric and the isotonic responses was exhibited by the character of their respective temperature dependence, uterine tension first increasing rapidly between 10° and 30°C. and then slowly between 30° and 40°C., the extent of isotonic shortening being unaffected in that range. Since these findings indicated that partial activation of the uterus was possible (i.e., it did not conform to the all-or-none law), it was apparent that the behavior of the myometrium with respect to hormones and inotropic substances could not be deduced from studies of the unloaded isotonically contracting muscle.

The relationship between load and work, exhibited by the characteristic parabola, provided a value for the maximal working capacity which stood in good agreement with that calculated from the tension measurements by means of Hill's formula—$(l \times t)/6$. Adding progesterone to a uterine strip

*in vitro* reduced the value of the "optimal load" and severely decreased the total work and the tension developed. Maximum isometric tension was always developed at the resting length, this correspondence being independent of stimulus strength. An added effect of progesterone, as compared to estrone, was to increase the stimulation threshold. A study of the kinetics of shortening as a function of after-load yielded a series of values of $dl/dt$ that conformed to Hill's "characteristic equation." Additional aspects of uterine contraction have been considered by Csapo & Corner (38, 39, 40), Schofield (177), Csapo (42), and Horvath (106). Recent investigations upon mammalian smooth muscles, other than the ileum and the uterus, include the studies of Bülbring *et al.* upon the taenia coli of the guinea pig (30, 31) and the rabbit's sphincter pupillae (32); and of Furchgott & Bhadrakom (65) on aortic strips. The pharmacology of vascular smooth muscle has recently been reviewed by Furchgott (66).

It is to be regretted that muscle physiologists have paid only scant attention to the perennial immunological bête noir, tissue anaphylaxis, and, in particular, to the immunologically induced contraction of smooth muscle. In the past this relationship has always been ascribed to the release of histamine by the antigen-antibody reaction and, indeed, striking analogies can ben drawn between their overt effects. But such similarities to anaphylaxis are not unique to histamine. To cite as evidence for the histamine theory the fact that both the specific and the histamine-induced contractions are palliated by antihistamine drugs is to fall into a logical trap.

In its simplest form, the basic question that needs clarification is this: how does the reaction of an antigen with antibody upon (or near) the surface of the smooth muscle set off a contraction? Specific serological precipitation leaves nothing in the supernate that is pharmacologically active. Views that the protein metabolism of the muscle becomes somehow disturbed as the result of its exposure to an antigen are not tenable, if closely scrutinized.

Sensitization of smooth muscle can be accomplished in a number of ways: (*a*) active sensitization, (*b*) passive sensitization, and (*c*) reverse passive sensitization. In the first instance antibodies are produced by the host; these are produced either in the reacting tissues themselves, or are manufactured elsewhere and migrate to the target organs. In the second case, the antibodies are produced in a separate host and upon transfer to a recipient are "fixed" to (or at any rate, are in equilibrium with) many tissues, including the "target organs." In the third case the antigen is infused and specific anaphylaxis (by challenge with antibody) can be demonstrated within the length of time needed to prepare the muscle. Although it has not been clearly demonstrated that passive sensitization can occur without a lag period, there is evidence that the period can be very short (158, 180).

The observation that tissues may be sensitized by soaking them in antibody is due to the past studies of Dr. D. H. Campbell (and to the continuing work of Terres & Campbell) who demonstrated that normal guinea pig gut

exposed to purified antibovine serum albumin (anti-BSA) can subsequently give specific reactions with, and then become desensitized to, BSA. Since it has still to be established that an antibody molecule having a molecular weight of about 160,000 can penetrate the muscle membrane under these conditions, it must remain that sensitization was accomplished in this case by some other process, probably by physical adsorption. In view of the physical dimensions of the antibody, and of the opportunity for the operation of secondary forces (such as H-bonds, nonpolar van der Waals attractions, etc.) between the antibody and the tissue, this is not unlikely.

During the studies of Penn *et al.* (146, 147) it was shown that reverse passive anaphylaxis could be induced by infusing (intra-arterially) nonsaponifiable liver fractions into guinea pigs and challenging the surviving guts with antibodies prepared against these fractions in the rabbit. It is unlikely that the antigen could have been the source of histamine under those circumstances.

A divergence with respect to histamine intoxication and susceptibility to anaphylaxis is observed in mice and rats, animals which have been classically regarded as being resistant to histamine. Campbell *et al.* (228) produced fatal anaphylactic shock in mice actively sensitized to BSA by challenging them with the specific antigen, and showed that mild sensitization could be developed in mice by the passive transfer of homologous mouse antiserum. Hochwald & Rackeman (102) demonstrated the active sensitization of rats to horse serum by Schultz-Dale tests. While the rat guts reacted specifically to the antigen their responses to histamine were inconstant, relaxation being observed as often as contraction. In certain instances complete refractoriness to large doses of histamine was observed. Campbell & Nicoll (227) showed that specifically shocked guinea pig lung can elaborate a material which produces contraction of rat uterus and, in view of the fact that the gut did not respond to high concentrations of histamine, concluded that this material was not histamine. Feigen & Campbell (229) failed to find a refractoriness to histamine in guinea pigs immunized with histamine-azo-ovalbumin. In addition, some recent experiments provided by Rocha e Silva & Rothschild (166) upon the persistence of strong skin anaphylaxis in the absence of demonstrable histamine in the skin, and the studies of Mongar & Schild (142) showing that anoxia and certain inhibitors of glycolytic and oxidative metabolism inhibit the "anaphylactic release" of histamine but potentiate its production by "48/80," present further doubts about the validity of the histamine idea.

The outlook for the resolution of the problem appears to be pretty dim, even in view of recently developed methods for the chemical estimation of histamine (124); because one cannot argue from negative evidence, the case against histamine must remain entirely circumstantial. The issue will be in doubt either until the participation of histamine (not merely its compresence) in the overall response is unequivocally established or until another mecha-

nism, not involving histamine, is found to obtain. At the present time it must remain as a challenging problem to the muscle physiologist, and such contributions as were made by Einbinder *et al.* (50) represent a refreshing approach.

(*b*) Cardiac muscle: The studies of Hajdu & Szent-Györgyi (78, 79) upon the mechanism of action of deoxycorticosterone and digitalis permitted them to develop an explanation of the "treppe" problem, based upon the accumulation of intracellular potassium during rest and its extrusion during contraction. These ideas were extensively discussed by Szent-Györgyi (192) and, in substance, are reviewed here. It is assumed that sufficient K is accumulated by a resting heart to depress the association between actin and myosin. A series of contractions, during which there is an accompanying reduction of intracellular K, restores the muscle to the "favorable" condition by increasing the tendency for actin and myosin to associate. In support of this hypothesis it is pointed out that a difference in [K] amounting to as little as 10 per cent of the total concentration can bring an *in vitro* system from complete dissociation to super-precipitation. The old concept of the heart hormone was revived by conceiving the existence of a steroid (AS) in the serum or liver [cf. Green (73)] capable of abolishing staircase by influencing the permeability of the heart-muscle membrane. The beneficial action of digitalis on the heart was thought to result from its high affinity for the heart, particularly in conditions involving a deficiency of the natural hormone. Antagonistic AS's, having an opposite effect to epinephrine and digitalis, were also considered. These studies were extended by Hajdu (80) with a view of obtaining precise information regarding the ionic shifts in the spectrum of events between staircase and contracture.

Hajdu measured the changes in the internal potassium and sodium under a variety of conditions that affected the "treppe" phenomenon. The height of developed tension was found to parallel the loss of K from the heart. Contracture appeared to depend upon a fixed relationship between the internal [Na+K] and the logarithm of the external [K], and it was inferred that contracture was determined by the membrane potential, as calculated from the intra- and extracellular [K]. A plot of [Na+K] against the calculated potential was, expectedly, linear and negative, from which the author concluded that with an increase of the total ionic content a higher membrane potential was necessary to prevent association of actomyosin, hence contracture.

In his discussion of the role of K in contracture the author points out that it has two antagonistic effects on tissue actomyosin: thus a decrease of intracellar K will augment the association of actomyosin while a decrease of extracellular K will diminish the association, and contracture will result if the equilibrium between the two is disturbed. He also proposed that under natural conditions (i.e., in an ionic environment favoring its association) the actomyosin is kept in a dissociated state by the static charge provided by the membrane potential. During excitation the "static" effect of the membrane potential is changed to the "rapid" effect with a consequent disturb-

ance of the dissociation of actomyosin. It is also assumed that since both effects are directed inward, the means whereby these two actions are propagated are different, the "rapid" effect producing a contraction and the "static" effect producing a permanent change.

On the basis that the "static" membrane potential could not produce a change in the internal ionic distribution that would tend to dissociate the associated actomyosin (every point inside the fiber having the same potential, hence there being no electrical field), it was conceived that the "static" effect was propagated inward by the pH change associated with a shift in the relative potassium concentrations across the membrane.

The view that these phenomena are accounted for by the pH change, incident to a shift of K across the membrane, might represent an insufficient condition; and in this regard the experiments of Feigen *et al.* (55, 56) and of Sutherland (188) upon the mechanical responses of the electrically driven rat ventricle and the associated changes in the internal K concentration that occur when the muscle is exposed to a variety of ionic permutations and temperatures, are particularly relevant.

One of the contributions of these studies was the demonstration that partial contracture and hypodynamia exhibited in a heart strip working for 15 to 20 hr. in an unchanged medium could be reversed by the addition of bicarbonate, either as the K or Na salt, all other constituents of the "reference solution" having no effect. Although this maneuver lowered the pH of the medium in the presence of 100 per cent $O_2$, other manipulations such as changing the gas phase to 95 per cent $O_2$-5 per cent $CO_2$ (which had been shown to increase the internal [K]) had no effect. The tentative hypothesis was developed that the factor principally determining the course of the contracture was the replacement of tissue bicarbonate with chloride, the supporting evidence being the appearance of a fine precipitate of $CaCO_3$ on the sides of the muscle chamber. The decline of activity, owing to the increase of internal chloride, is consonant with the observations of Sarkar (174) in which it was found that the increase in chloride binding of actomyosin would tend to shift its isoelectric point toward the acid side and hence reduce its combination with ATP. If these results are applicable to the heart under the circumstances described, then it can readily be seen that the heart will go into contracture since the plasticizing action of ATP would be reduced.

The ventricle strip has certain properties which make it useful for biophysical studies. In the rat, the right ventricle is thin enough to permit adequate diffusion, and it has very little connective tissue. Hence responses to environmental changes take place quickly and represent, in a large part, the reactions of the contractile substance rather than of its supporting structure. Two interesting features deserve comment: one of them is the response of the static tension to temperature, and the other is the relationship between static and developed tension.

The dependence of static tension on temperature in the rat ventricle (56) did not show the monotonic function described by Szent-Györgyi (190).

When the muscle was set at 0°C. against a tension of about 0.50 gm., an increase in temperature produced a rapid rise in tension which became maximal at about 10°C.; thereafter it fell quickly to a minimum at 27.5°C., whence it rose in sigmoidal fashion, becoming flat in the region 40° to 45°C. It was not possible to obtain a response, using the maximum output of the stimulator, in the region below 10°C. When the tension was brought down by an increase of temperature, stimulation became effective and the contractions increased to a maximum at 27.5°C., coincident with the point of minimum resting tension. As the temperature was increased the contractions declined, the value at 37°C. being 60 per cent of that observed at the lower temperature. As the static tension peak at 10°C. was passed, there was a definite qualitative change in the elasticity of the tissue. Below 10°C. the muscle was quite plastic, and the response to a deforming force took place very slowly, but, when the peak was passed, the tissue became "elastic." A possible explanation for both of these effects can be based upon a mechanism similar to that proposed by Polissar (q.v.). Thus the increase in static tension in the lower ranges of temperature could result from the effect of heat on one order of rearrangement (possibly intramicellar) while the decrease in tension at higher temperatures could occur from an intermicellar rearrangement. In the latter case it could involve a more effective coupling with ATP, the reduction of the muscle's tension being an overt expression of its plasticizing effect.

The rates of activity decay measured at 27°, 37°, and 45°C. appeared to follow first order kinetics but their rate-constants did not form a straight line in a plot of their respective logarithms against 1/T. Activation energies calculated between 27° to 37°C. and 37° to 45°C. were 26,820 and 44,500 calories, respectively, the latter value suggesting protein denaturation.

Work curves were typical parabolas. A study of the work output at various frequencies of stimulation at 27°C. and 37°C. was made by Sutherland *et al.* (187). The optimal frequencies were 140 and 140 to 200 per min. for the 27° and 37°C. categories, respectively, the corresponding rates of work production being 4.4 and 2.8 gm. cm. per min. The difference between the magnitudes of maximum work rate result, of course, from the fact that the amplitude of contraction at 37°C. is proportionally lower; the reason for this is not known but it is fairly certain that one of the contributing factors must be the more rapid decay of the active state at the higher temperature. Studies by Szent-Györgyi (189) on the tension-temperature relationship of the rat's diaphragm present an astonishingly close correspondence with the results described for the rat's ventricle, as in both cases the maximum tension was developed at 27°C. The fact that only a fraction of the total energy potential is being utilized at body temperature may be a significant feature in the design of biological systems and in their economic behavior. These ideas are admirably developed by the author.

Garb & Scriabine (67) found that the inotropic response of the cat's papillary to glucose was greater at 27° than at 37°C. Studies of the inter-

mediary metabolism of the driven rat's ventricle in relation to the action of drugs were made by Masuoka & Saunders (130) and Masuoka *et al.* (131). The oxygen consumption of the working rat's ventricle was studied by Reiter (157) while that of the cat's papillary was measured by Lee (121). Species differences in the utilization of glucose by the heart were reported by Garb *et al.* (68). The behavior of cardiac glycosides with respect to cations was studied by Schatzmann & Witt (176), Witt & Schatzmann (223), and Willbrandt & Caviezel (222). The inotropic response of substrate-depleted rat ventricle strips to *k*-strophanthoside and glucose was studied by Feigen *et al.* (57, 58) in Ringer's solutions anionically modified by substituting $NO_3$ and SCN for chloride. Substrate-depleted strips failed to respond to strophanthoside until glucose was added, and the response to either substance was greater in the modified than in the normal Ringer's. The effects of temperature and cardiac glycosides in producing incomplete tetanus were studied by Whitehorn (219) on rat- and frog-ventricle strips.

It is evident that a good deal of the confusion concerning the action of drugs could be resolved if there were precise knowledge concerning the behavior of the tissue studied. The process of screening amounts to the study of the special case, and while the predictive attributes of such information may be very high, it can operate to becloud understanding.

*The "active state" of muscle.*—Shortly after excitation the contractile elements undergo an alteration of state, from rest to activity, which makes it possible for them to develop maximum tension. This state has a definite time-constant that is determined by a variety of conditions, among them temperature [Macpherson & Wilkie (126); Ritchie (161)]. Because of the architectural arrangement of the contractile and series-elastic elements with respect to the framework, the true time-course of the contraction is obscured by the interval necessary for the contractile units to take up the slack of the series-elastic elements. The active state having decayed at a definite rate (after an initial plateau) since the onset of activity, contractile function is over before its full effect has been transmitted to the fiber as a whole. If the necessity for taking up slack is avoided by an earlier coupling, the external tension manifested becomes greater. This can be accomplished by applying a quick stretch to the muscle shortly after an effective stimulus (69), or by restimulating it in such a way that the time of the second shock falls when the series-elastic elements have been maximally lengthened by the first one. Hill (94) showed by means of quick-stretch experiments that the active state begins to decline from its plateau before the tension of an isometric twitch becomes maximal and that the active state is set up very suddenly (within one-tenth of the time necessary to attain maximum twitch tension). Owing to the speed with which the active state was developed after a shock, it was considered unlikely that diffusion could be involved in the process of its inward propagation. Since the quick-stretch method was not sensitive enough to yield precise values, Hill (98) used the tension-decline of a muscle released at constant

speed as a means of measuring the end of the plateau. According to this method, the decay of the active state begins at about 0.4 to 0.5 the time required for the attainment of maximal twitch tension.

Macpherson & Wilkie (126) estimated the duration of the plateau by measuring the time at which the twitch- and tetanus-tension curves of an isometrically contracting frog sartorius began to diverge, and they found that the end of the plateau occurred at about 44 msec. Ritchie (160) estimated the duration of the plateau in the rat's diaphragm to be 5 to 7 msec., as based on the time between stimuli in an isometric tetanus. Studies on frog's muscle at 0°C. by the same author (159, 161), using the onset of tetanus-tension decline as a measure, showed the tension to drop at 35 msec. after the stimulus. The extent of the plateau was calculated by subtracting the latent period (10 msec.) for a single twitch, and its duration was found to be independent of the muscle length. The relationship between temperature and (log) time of relaxation onset was linear; calculations based upon this relationship showed a $Q_{10}$ of 2.2 for the duration of the active state. The fusion-frequency in frog muscle was found to be 40 shocks per sec., which agreed well with the value of 25 msec. for the duration of the plateau.

Prior studies, made by Sandow & Mauriello (169) on the relationship between fusion-frequency and temperature, indicated a value of 40 msec. for the full duration of the active state at 2°C., which stood in good accord with Hill's estimate; these values declined at higher temperatures and were found to be 11, 4.5, and 3 msec. at 10°, 20°, and 30°C., respectively. It was also suggested in this paper that there might be a connection between the twitch-potentiating property of the nitrate ion and its effect upon the active state, the views being based on the force-velocity evidence at 0°C. (170) wherein the ratios $b/l$ and $a/P_o$ were increased in nitrate, with respect to chloride, but in which the contractions were not affected.

Before discussing the nature of the active state other correlates of the precontractile and early contractile phases should be noted. D. K. Hill's light-scattering experiments (101) show structural rearrangements to take place during the beginning of the latency relaxation; and from A. V. Hill's work (97, 99) it is obvious that profound physico-chemical changes occur before contraction is well underway. His results showed that the heat production is already maximal when the contraction is just starting, and disappears when the active state has ended.

It is quite evident that Sandow's correct appraisal of the nitrate effect exerted a profound influence upon subsequent studies of the active state, as well as on the ideas concerning the relationship between the contractile events and those occurring at the fiber's surface [E-C coupling, Sandow (168)]. On account of its significance, some of their earlier work on abnormal anions will be briefly reviewed. Basing their studies on Chao's results (34) with "abnormal" anions, Kahn & Sandow (113) investigated the electrical and mechanical concomitants of muscular response upon frog sartorii, surviving in chloride- and nitrate-Ringer's. The effect of nitrate ion was to de-

crease slightly both the latent period and the latency relaxation, to increase the depth of relaxation, and profoundly to potentiate the twitch-tension so that its magnitude approached that of a tetanus. Since the stimulus strength was supramaximal and the action potential was not affected by nitrate, the observed increase in twitch-tension could not have been attributed to fiber recruitment or to tetanization; hence, it was concluded that each fiber was producing a tension approaching tetanus strength. From the fact that these changes occurred so rapidly, probably before the nitrate ion had time to penetrate the interior of the fiber, it was inferred that its effect must be on the membrane; and, because of the effect on the precontractile events, that it acted on the excitation-contraction coupling mechanism. An additional account (114) from this laboratory showed that the twitch duration and mechanical response were increased in a regular order with the Hofmeister series, i.e., $Cl < Br < NO_3 < I$.

A study of the effects of abnormal anions ($NO_3$, Cl—SCN, $NO_3$—SCN) upon the mechanical response of the electrically-driven rat ventricle to temperature, glucose, cardiac glycosides (57, 58) and various cations has been made in the reviewer's laboratory. Since the heart strip does not go into tetanus it is not possible to use this approach as a means of studying the active state; however, inferences may be drawn from the rate of onset of the effect. The increase in force of the individual contractions and the rate of rise to a maximum value, after adding glucose and *k*-strophanthoside in the presence of nitrate ion, are much greater than in chloride (57). Similar differences are observed in mixtures of thiocyanate and chloride, and thiocyanate and nitrate.

The effects of nitrate and caffeine were examined by Ritchie (162) on the active state of the frog's sartorius at 0°C., its intensity being determined by the quick-release method and its duration by the method of post-tetanic tension decay. The duration of the active state under these conditions appeared to vary with muscle length. Both nitrate and caffeine prolonged the active state as well as the duration of the plateau, the increase in twitch-tension observed being attributed to these effects. The tetanic tension was reported reduced by nitrate but not consistently affected by caffeine.

The effects of nitrate, iodide, and bromide were studied on the duration of the active state by Hill & Macpherson (100). Their exhaustive study showed the tension, duration, and heat production of a twitch to be increased in the order $Br < NO_3 < I$. Since none of these parameters was affected in the tetanized muscle, the experiments confirmed Sandow's hypothesis that the potentiating effect of the "abnormal" anions was attributable to their prolonging the active state, and that the heat and tension increase in a twitch were consequent to this action.

The isotonic shortening of the after-loaded frog sartorius was shown to be augmented in a regular way as the proportion of nitrate to chloride was successively increased. Although the tension was found to be increased with iodide it was not possible to use this anion in proportions of more than 70 per

cent in the mixture because of irreversible damage to the muscle. At all temperatures the duration of the twitch was found to be longer in nitrate than in chloride, and the character of the temperature dependence became somewhat different. The increase in twitch tension as a function of resting length was greater in nitrate or iodide than in chloride. The load-velocity relationships of tetanized muscles, surviving either in nitrate or iodide solutions, were identical over the entire range of observed shortening velocities, and in all cases the results conformed to the normal force-velocity function. Myothermic studies showed a doubling of the twitch heat in nitrate or iodide; but, as compared to chloride, these anions produced only an insignificant increment in the heat generated by a tetanus. The rate of onset of the nitrate or iodide effect was measured, and the time of one-half increment was shown to be 40 sec., and that for one-half decrement, 90 sec. Basing their argument upon a probable value of the diffusion constant for iodide, the time-tension and time-concentration functions could be practically superimposed, both for the onset as well as for the retreat of the effect.

Measurements made for these authors by Abbot showed the rate of loss of radio-iodide from an iodide-equilibrated muscle (exposed to chloride-Ringer's) to have a half-life of 90 min. for outward diffusion. By making suitable corrections for its entry into a chloride-equilibrated muscle, it was calculated that only about 0.5 per cent of the amount would enter in 40 sec., at which time the observed tension-increment was already at one-half of its maximal value. On these grounds any direct reaction of the anions with contractile elements was considered improbable and it was hence concluded that the effect of the anion must be mediated via its influence on the membrane.

The studies of the active state of muscle and, in particular, the recent contributions regarding the modification of its time-course by anions, made by the groups in New York and London, have provided a "new look" at the relationship between excitation and response. This is best put in the words of Hill & Macpherson (100):

> Hitherto one has tended to regard the excitatory process at the surface as pulling a trigger that fires off an independent contractile process inside, which then runs its own course, up and down, to completion. This idea is now seen to be wrong. The duration of the contractile process inside is determined, over a fairly wide range, by something which does not occur inside but at the surface.

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# RESPIRATION[1,2]

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## Regulation of Respiration

*Respiratory centers.*—The neurophysiology of the reticular formation has developed rapidly in recent years. Its general results should be of interest to those concerned with central respiratory regulation because of the obvious analogies between reactions in different functional systems. Thus, reciprocal and nonreciprocal inhibition and facilitation of spinal motoneurons in response to stimulation of medial and lateral areas in the bulbar reticular formation (120) may have some relation to the phasic and tonic respiratory responses elicited from these areas. Liljestrand (172) regards the respiratory responses obtained upon stimulation of the medial reticular formation as instances of general reactions of the facilitatory area of Magoun. This area would possess some degree of topographic specialization, the ventrocaudal part facilitating inspiration, the dorsocranial part expiration. A considerable overlapping of vasomotor and respiratory regions can be demonstrated (7) by exploration of the medulla with stimulating electrodes. All this supports the concept of central respiratory areas as "semispecialized portions of a widespread reticular system that provides a common denominator of integration, mediating the harmonious coöperation of motor activity in somatic, autonomic and respiratory spheres" (148).

Gesell *et al.* (122) have tried to explain respiratory regulation, regarding it as a general example of motor integration, by combining concepts on reciprocal interlocking of antagonistic nerve cells with views on centrogenic and reflexogenic generation of neurocellular current. Elementary explanations for the rhythmicity of breathing and for the integration of central respiratory drive are harmoniously linked together in this theory. The existence of a specific coördinating respiratory centre in the bulb has been questioned (172). Respiratory integration may take place at all levels down to the spinal motoneurons. Respiratory responses obtained from higher parts of the brain were reviewed by Poirier & Shulman (214) and Christian *et al.* (68). The latter note the importance of supramesencephalic regulations in connection with certain forms of behaviour and other work. Segundo *et al.* also consider the respiratory effects obtained from the fornix and the wall of the third ventricle as part of a complex pattern related to other functions (235).

The question whether spontaneous breathing requires the existence of a

[1] This review covers the period from approximately July, 1954, to April, 1955.

[2] The following abbreviations, not explained in the text, are used in this chapter: Aa-gradient (alveolar-arterial difference); $D_{CO}$ (pulmonary diffusing capacity for carbon monoxide); $P_{O_2}$ and $P_{CO_2}$ (oxygen and carbon dioxide tensions).

[3] It is a pleasure to acknowledge the technical assistance of Geertje van der Veen.

general background of afferent stimulation results anew from the experiments of Breckenridge & Hoff (44), showing the relationship between reflex respiration, evoked by afferent impulses in conditions of apnea, and subsequent patterns of spontaneous breathing. Transmedullary electrical stimulation may likewise arouse slumbering breathing mechanisms (43). During progressive pentobarbital anaesthesia, local medullary electrical stimulation (139) can also evoke respiratory responses after arrest of the more sensitive spontaneous rhythm. Here, as well as in transection preparations (43), the pattern of the electrically-elicited responses reflects the progressive breakdown of the mechanism concerned with the coördination of respiratory movements. This mechanism, activated by afferent or direct electrical stimuli, may under improving conditions change into an intrinsic self-cycling mechanism, apparently independent of extramedullary influences.

The pattern of this intrinsic rhythmicity depends upon the available neuronal substratum. Certain levels of respiratory integration can be recognized at ascending levels of brain stem transection. Hoff & Breckenridge (148) have given a systematic description of the various patterns encountered in numerous experiments of this kind. The irregular, ataxic, abrupt breathing of the medullary animal has been called medullary eupnea, and is considered as the fundamental rhythm of normal quiet breathing. A sighing "all or nothing" respiration, observed at medullary but more fully at supramedullary transections, as well as various forms of periodic breathing, depend on a second intrinsic slow rhythm of the brain stem, normally suppressed in favour of the faster eupneic rhythm. Apneustic breathing, Biot's breathing, and Cheyne-Stokes' breathing are explained as interactions of the elementary fast and slow rhythms, modified by inhibitory and facilitatory systems. In other forms of Cheyne-Stokes' breathing the delay in lung-to-head circulation time is an important feature of a self-perpetuating cycle, involving periodic changes in oxygen saturation (126). Oxygen, carbon dioxide, and aminophylline may restore regular rhythm.

Wyss (273) sees rhythmic activity as the result of the interplay of several neurons scattered through the bulbo-pontine reticular formation. Intrinsic processes of facilitation and inhibition, assisted by vagal inhibitory impulses, convert the primary inspiratory tonus to rhythmic activity. Baxter & Olszewski (17) have found that stimulation of the region of the pneumotaxic centre resulted in characteristic inspiratory, and not in the expected expiratory, responses. This poses the general question as to the significance of stimulation and destruction experiments with respect to normal function.

The atlas of Olszewski & Baxter (203) on the cytoarchitecture of the human brain stem provides a morphological basis for the analysis of its function. As in the cerebral cortex such an analysis probably will involve more than the solution of a jig-saw puzzle of localized functional units.

*Afferent and efferent respiratory impulses and respiratory reflexes.*—Vagal connections in the medulla could be traced through the solitary tract and in the reticular formation closely ventromedial to this tract. Effects, obtained

far caudally, suggest that some respiratory vagus arcs do not pass through the medullary "centre" (172). Action currents, indicating efferent respiratory activity, could be derived only from the motoneurons of the nucleus ambiguus after bilateral vagotomy (1).

The principle of centrifugal control of sensory messages, so well established in muscle and some special sense organs, has received little explicit attention in the proprioceptive control of respiration. The diverse types of pulmonary vagal afferents and their peripheral receptors have been further analyzed. Slowly-adapting stretch receptors are located mainly in or very near to large intrapulmonary bronchi. Their activity is related to bronchial tone, since it is affected by bronchoconstrictive drugs (264). Similar receptors in the extrapulmonary bronchi are stimulated by deflation of the air ways. Paintal (209) has detected readily-adapting specific pulmonary deflation receptors of a new type, situated in the pulmonary capillary region. Their normal function probably consists in inducing a reflex respiratory acceleration. Vagal afferents from gastric stretch receptors and other gastric and intestinal drug-sensitive receptors, located by an injection-response time method (208), have been mistaken for pulmonary fibers in the past (207). Indirect effects of drugs should be distinguished from the direct effects upon receptors. Thus, 5-hydroxytryptamine (serotonin), which stimulated the specific deflation receptors (209), did not stimulate pulmonary stretch receptors when administered in small doses. With larger doses the concomittent bronchoconstriction may cause indirect stimulation (161). A review on reflexes from stretch receptors in blood vessels, heart, and lungs has been presented by Aviado & Schmidt (10). The pharmacology and the respiratory effects of serotonin have been reviewed by Page (206).

Wyss (274) holds the fast-conducting stretch afferents responsible both for the expiratory and the inspiratory Hering-Breuer effect. Inhibition of the inspiratory innervation is associated with a high frequency discharge at increasing lung volume, facilitation with a lower frequency discharge at decreasing lung volume. Frequency-dependent effects of vagal type have been observed also by Liljestrand during medullary stimulation (172). Gesell *et al.* (122) have studied the rôle of several respiratory afferents, notably tonic inspiratory responses to carotid nerve stimulation after vagotomy. Some of the effects depend on the exact timing of stimulation during the respiratory cycle.

Bucher (51) could not establish the reflex character of the compensatory chest wall expansion in acute pneumothorax, in spite of earlier experiences with vagotomized rabbits (52). The results of Heaf & Prime (143) are in accordance with the fundamental mechanical facts, outlined by Fenn (138).

A nonspecific conditioning influence of tonic vagal impulses on the reflex activity of the centre and on respiratory frequency has been demonstrated in normo- and hypothermic guinea pigs (127). A curious effect, mediated by the vagus nerves, is the prolonged survival of the asphyctic respiratory centre by inflation of the lungs or by vagal stimulation (248). The afferent and

vegetative functions of the phrenic nerve and its anastomoses are surveyed by Drouet *et al.* (99). Reflexes arising from the nasal mucosa have been analyzed by Massion *et al.* (184).

*Chemical regulation.*—Chemical responses to local injections of bicarbonate have been elicited from the lateral reticular formation. The effect of phosphate buffers of pH 6.98 was minimal in comparison to that of bicarbonate (172). Direct pH determinations during acute ischemia or anoxia (257) and during asphyxia and hyperventilation (260) disclosed large and fast changes in the cerebral cortex. A decrease of 0.4 pH units was reached after complete ischemia of only 2 min. duration.

Central cholinesterase activity in connection with the anticholinesterase properties of acids is considered by Gesell *et al.* to be a primary factor in the control of breathing. Anticholinesterase injections into the vertebral artery caused hyperventilation (122). Variation of enzyme activity in regulatory cells might well constitute a factor in adaptation processes.

The composition of the cerebral ventricular fluid is in some respects representative of the condition of the centre. Metabolic acidotic changes in the blood, in contrast to respiratory acid-base shifts, are only slowly and incompletely reflected by concomittant changes in the intraventricular fluid. This seems to bear on the question why the respiratory response is more pronounced when a given decrease in blood pH is caused by $CO_2$-inhalation than when it is produced by the injection of acids (23).

The lag of changes in ventilation behind rapid changes in arterial $P_{CO_2}$ leads Grodins *et al.* (131) to the concept of tissue concentration as the controller input in their feedback theory of respiratory regulation. This supplements other evidence, collected in last year's review by Rahn, for the importance of tissue $P_{CO_2}$ above arterial $P_{CO_2}$ as the factor determining the central response. In consequence the conditions governing gas exchange between the chemosensitive central regions and the blood, and their eventual regulation, become important factors in the regulation of breathing and in acclimatization. In the explanation of the slight hyperventilation produced in man by oxygen at atmospheric pressure the responsible factor is thought to be a hampered transport of $CO_2$ from the centre, resulting from an insufficient reduction of oxyhemoglobin (239). The raised intracranial pressure occurring in emphysematics is probably attributable to a vasodilator effect of anoxia and hypercapnia on the cerebral vessels (262).

A reduction of carotid body blood flow by electrical stimulation of the cervical sympathetic nerve, possibly leading to an intrinsic anoxia, has been demonstrated by de Burgh Daly *et al.* (89). This suggests the possibility of an efferent control of carotid body stimulation. The increased chemoreceptor discharge caused by certain drugs injected into the common carotid artery may equally result from an action on the superior cervical ganglion, rather than from a direct action on the carotid body (88). Reviews on the physiological and pharmacological properties of the carotid body chemoreceptors (144) and on visceral receptors (145) have been presented by Heymans & de Vleeschhouwer.

## Respiratory Adaptation

*Acid-base shifts.*—In acute disturbances of respiratory origin renal mechanisms (96, 256) are not sufficient by themselves to explain the acute shifts in blood bicarbonate. A redistribution of electrolytes across the boundary of the readily available extracellular fluid compartment has been observed (125).

The increase of tubular reabsorption of bicarbonate-bound base in chronic respiratory acidosis is significantly greater than that observed in the acute form. An adaptation, involving the kinetics of the reactions concerned, is implied (250). Studies on the influence of carbonic anhydrase inhibition upon renal bicarbonate reabsorption may add further insight as to the factors regulating bicarbonate excretion (180, 253). Alterations in plasma $P_{CO_2}$ rather than in pH seem to be normally responsible for changes in bicarbonate reabsorption (253). The effect of high doses of acetazoleamide (Diamox) in man on alveolar and blood gas tensions and on ventilation has been investigated and was found to be more pronounced under hypoxic conditions (258).

*Central adaptation and acclimatization to high altitude.*—Christensen, in an excellent review on respiratory regulation (138), finds the mechanism of control during hypoxia still more or less an unsolved problem. Earlier work seems to indicate that in severe acute hypoxia the $CO_2$-stimulus does not contribute to the maintenance of ventilation. Direct information as to the actual tissue $P_{CO_2}$ of the centre is, however, not available. In acute hypoxia of moderate degree the hyperventilation begins to develop only after at least 1 hr. The primary mechanism of this process is uncertain. After elimination of chemoreflexes by oxygen breathing a "fixed" hyperventilation is still present during exercise in acclimatized subjects. This persists even for several days after return to sea level. It is not fully explained by the decrease in blood bicarbonate but must result from an increased sensitivity of the centre to $CO_2$ (67). Equally, the respiratory adaptation to high levels of $CO_2$ in emphysema involves a diminished ventilatory response, which is only partly attributable to increased bicarbonate (218). A detailed analysis of the respiratory sensitivity to $CO_2$ and pH in various clinical states of hypercapnia, anoxia, and acid-base derangement seems to confirm the hypothesis of a specific carbon dioxide adaptation in prolonged hyper- and hypocapnia. The decreased sensitivity to $CO_2$ was observed not only in chronic respiratory acidosis but also in chronic metabolic alkalosis where it may persist for even four months after recovery (4).

Current knowledge on the respiratory features of high altitude acclimatization has been presented by Riley *et al.* (138). Many problems of high altitude have been dealt with in a discussion by physiologists (92), some of whom were active in the 1953 Mt. Everest expedition (147). The arterial oxygen saturation in acclimatized subjects during rest is not much higher than in unacclimatized men at the same altitude (185). The use of oxygen apparatus by acclimatized men has been found desirable above 24,000 ft. (40). After breathing oxygen from closed set apparatus at altitudes from 18,000 to

24,000 ft., the transition to air breathing at the end of a day caused hypoxic symptoms lasting for about 2 hr. (40). Pugh (219) comments on the use of the open set apparatus, which proved indispensable on Mt. Everest above 26,000 ft. In experimental conditions oxygen may reduce high altitude hyperventilation during work by 20 to 40 per cent. In spite of the corresponding rise in alveolar $P_{CO_2}$, the dyspnea was greatly relieved. Progressive anoxia (246) and high altitude deterioration (186, 261) have been described, sometimes in dramatic detail (275).

The nutritive and rinsing function of the circulation in the rabbit brain is improved in the acclimatized state (204). Blood hemoglobin may increase 0.13 gm. per cent per day during acclimatization, yet there is no obvious relationship between hemoglobin level and physical performance (220). In rats polycythemia may have some favourable effects (170). Bleeding experiments in mice however show that this is not the only factor (134). High altitude polycythemia is attributable entirely to an increase of red cell formation. Red cell life span was found to be normal in Peruvians born and living at altitude (20), as well as in acclimatizing subjects taken from sea level (225).

*Exercise.*—During severe exercise the ventilatory response to a given level of arterial $P_{CO_2}$ is greatly increased. This can be sufficiently explained by an accumulation of lactate, a rise in body temperature, and a stimulus connected with want of oxygen (12). Similar conclusions were reached in other studies (77) with less severe exercise. In steady state exercise, however, the effect of $CO_2$ inhalation in combination with exercise was such that one factor tended to lessen the action of the other (80). Means of characterizing responses to conditions tolerable only for short periods deserve attention.

Inhalation of 66 per cent oxygen delayed the exercise breaking point. The concomittant rapid reduction of ventilation and rise in alveolar $P_{CO_2}$ were ascribed to the abolition of an arterial anoxemia (11, 219). An adverse effect of pure oxygen was noted during severe exercise. This was probably caused by the dilating action of excess $CO_2$ on cerebral vessels, which nullified the vasoconstriction which protected against the effects of high oxygen at rest (11). The dangers of insufficient carbon dioxide absorption when oxygen is breathed during underwater swimming are stressed by Donald & Davidson (94), in a study on the oxygen uptake of fin swimming divers. Narcotic levels of $P_{CO_2}$ may be reached without any warning respiratory symptoms. Schaefer (233) has found that a fixation of the pulse rate may precede symptoms of oxygen poisoning and also mentions a remarkable type of dyspnea in underwater swimming.

Kao & Ray (156) observed a rectilinear relationship between total ventilation and oxygen consumption during induced muscular work. Pulmonary circulatory responses to exercise have been extensively studied by catheterization techniques in patients as well as in normal subjects (53, 156, 243). Pulmonary resistance decreased (243) and the diffusion capacity of the lung increased up to a certain level (162). The Aa-gradient did not increase significantly in normal subjects, but pulmonary arteriovenous shunting decreased (15).

Steady states during exercise were attained in man within 30 min. (77), in decerebrate dogs after only 5 min. (157). In man, however, the plateau reached at this time is a transient one (80). A steady cardiac output is normally reached after 10 min. but under pathological conditions it occurs later or is never reached (53). In patients with rheumatic heart disease, however, steady states of arteriovenous $O_2$-difference and cardiac output were observed after only 2 to 3 min. (93). The second wind, or second steady state, and the dead point have been explained in terms of circulatory and thermal adaptation to exercise (230). In decerebrate dogs the regulation of exercise hyperpnea is unchanged. Cortical irradiation apparently is not an important factor (157).

*Hyperthermia and hypothermia.*—Both central and peripheral temperatures contribute to the initiation of thermal panting. The central stimulus is the more important one (173). Part of the exercise stimulus is attributable to hyperthermia (12, 13, 77).

Winterstein (269) advocates the use of the $C_{OH}/C_H$ ratio instead of pH in defining acid-base equilibrium at abnormal temperatures. In this sense a "normal" pH during hypothermia still indicates an acidosis. Fleming (113) discusses the problems of measuring blood pH at low temperature. Data as to the constancy of arterial $P_{CO_2}$ during cooling are somewhat conflicting (81, 176). The need for adequate artificial ventilation in the prevention of fatal acidosis is however recognized (112, 176). The increased solubility of $CO_2$ at low temperature is an important factor. The relative increase in ventilation with inhalation of 6 per cent $CO_2$ has been found the same at body temperatures of 38°C. and 26°C. (81). Other investigations point, however, to a decrease of the regulatory function of $CO_2$ during hypothermia (176). Total ventilation was more than halved at 26°C. (81). Chemoceptor reflexes afford little protection against hypoxia in hypothermic cats (198). Apneustic breathing has been observed in cats and rabbits at body temperatures between 18° and 10°C. even with intact vagus nerves (16).

*Drugs and hormonal effects.*—It is only possible to list here work pertaining to cholinesterase activity in the centre (59, 105, 122, 272), which may throw some light on the question of central neurohumoral function. The intraventricular administration of autonomic drugs (109), substance P (106), and ammonium chloride (24) is of interest in view of the gradual and long-lasting effects. Epinephrine has a centrally stimulating action both by cerebral ventricular perfusion (117) and by intravenous infusion (41). Salicylates (3) and steroid hormones (175) increase the sensitivity to $CO_2$ by unknown mechanisms.

*Fetal and neonatal respiration.*—The ontogenesis of fetal respiratory regulation and the histology and function of the neonatal lung are reviewed by Rees (224). A synopsis of the importance of hypoxia in fetal and neonatal life, given by Cross *et al.* (84), covers such topics as the onset of ventilation at birth, the sensitivity to $CO_2$ and low $O_2$ in different phases, fetal hemoglobin and oxygen transport, and anaerobic metabolism. The infantile respiratory centre is more sensitive to $CO_2$ than the adult one (83, 192). This

hypersensitivity develops within a few hours after birth (192) and can be depressed by low oxygen (82). The complicated effect of hypoxia in infants (84) consists mainly in a poorly maintained hyperventilation, eventually followed by hypoventilation. Periodic breathing during hypoxia appears only at least 24 hr. after birth (192). Blood gas data at and after birth are scarce (129). The fetal respiratory centre can withstand protracted asphyxia (103).

Oxygen transport through the placenta of man (19) has been shown to depend in part on the excretion of fixed acid and carbon dioxide, causing a favorable shift in the dissociation curves during exchange. In sheep, the diffusion capacity of the placenta has been determined (163). Dawes *et al.* (91) present an excellent study on the fetal circulation in the lamb with diagrams for the relative distribution of blood flow and for the oxyhemoglobin content in the principal fetal vessels. The lifespan of fetal erythrocytes is significantly reduced at birth as a result of a sudden hemolysis occurring after relief from hypoxia (151). Other studies concern the mechanics of breathing (188) and pulmonary water loss (152) in newborn babies.

## Gas-Transport

*Ventilation and lung volumes.*—Current knowledge of alveolar air and its sampling has been presented by Rahn (138). Practical aspects of the volume and partial pressure of respiratory gases at altitude have been considered by Boothby *et al.* (138).

At tidal volumes of as little as 60 cc., detectable quantities of inspired helium penetrate into a space which cannot be washed out by the next deep expiration. This revives the concept of a cone-shaped boundary between alveolar and dead space gas (48). The importance of the time factor and of gas mixing by diffusion seems to follow from the interesting experiments by Roos *et al.* (227) on nitrogen clearance at various breathing rates and volumes. The meaning of dead space is discussed, and new arguments for the existence of a "stratified" inhomogeneity are included. Residual volume may be greatly underestimated by the conventional determination, since the important terminal phase of helium washout shows a very poorly aerated "slow" space, even in normal subjects, but much more so in emphysematics, where it may occupy 90 per cent of the functional residual capacity (146). In normals the "slow" alveolar space becomes smaller and more slowly ventilated in the supine position (33).

Normal standard values have been reported for intrapulmonary gas mixing and lung volumes (199). Predictions of residual volume from anthropometric and radiological measurements are worthless in individual cases (165).

*Gas exchange.*—A number of studies center around problems of gas exchange and respiratory quotient in connection with unsteady states. Changes of respiratory variables during pentobarbital anaesthesia have been analyzed with the aid of the $P_{O_2}$-$P_{CO_2}$ diagram, which allows a study of venous gas

tension as a function of ventilation and circulation (252). Using the same diagram, Farhi & Rahn (107) show how the over-all exchange ratio results from the metabolic and stores-exchange ratios and present a method for calculating changes in gas stores of the body for any changes in ventilation, cardiac output, and inspired gas tensions. The half-time of change of gas stores was 4.2 min. for $CO_2$ and 0.5 min. for $O_2$. A much slower change in tissue $CO_2$ would occur in the presence of a fixed artificial ventilation, but similar time constants have been found with intact ventilatory regulation (131). The accumulation of $CO_2$ during the intervals between intermittent oxygen insufflation has been studied in apneic dogs, in view of the potential applicability of this procedure as a means of mass resuscitation (111).

Fenn & DeJours (110) present an elegant method for analyzing breath-holding curves of alveolar gas composition, which provides a value for the oxygenated or virtual venous $P_{CO_2}$ and is theoretically applicable to the determination of cardiac output.

Hatch & Cook (142) define an over-all transfer coefficient for inert gases, analogous to the conductance of three resistances in series: ventilation, diffusion, and a factor depending on blood flow. The solubility of a gas determines on which of these factors its rate of absorption is primarily dependent. A practical procedure for calculating effective rates of ventilation, diffusion, and blood flow, using as test gases ether, acetylene, and carbon monoxide, has evolved from the mathematical analysis.

Forster *et al.* (115) have re-examined the theory of CO uptake from the lungs after a single inspiration and during "steady state breathing." The local variation of the diffusing capacity with alveolar ventilation and volume makes it difficult to define and to determine an over-all pulmonary diffusing capacity. With the steady state breathing method the fractional CO uptake will be influenced predominantly by the diffusing capacity of overventilated parts. With the breathholding technique the unequal distribution of diffusing capacity per unit alveolar volume betrays itself by a nonexponential decline of the mixed expired alveolar CO concentration with increasing time of breathholding (116). Thus $D_{CO}$ apparently decreases with increasing time of breathholding. The fact that the "apparent" total D is influenced by factors other than the diffusing capacity of the lungs must be remembered when differences in this measurement are interpreted in terms of pathological change. With a rebreathing method, Kruhøffer (162) did find an exponential decline of CO concentration in most normal adults. The attainment of a dynamic lung-bag equilibrium required only four breaths. The values for $D_{CO}$ were inversely related to the concomittant alveolar $P_{O_2}$. A correction method for these back pressure effects has been pointed out. Wilson *et al.* (267) estimate the necessary correction at about 10 per cent. Bartels *et al.* (14) have calculated the amount of arteriovenous shunting during inhalation of 35 per cent oxygen and air to be nearly the same.

The variation of the ventilation-perfusion ratio from one lobe to another has been determined by a direct sampling technique (232). The blood sam-

pled through a wedged catheter from an occluded pulmonary vein was almost identical to systemic mixed venous blood, whereas the $P_{O_2}$ in blood from an occluded pulmonary artery exceeded that in the systemic arterial blood by 20 mm. Hg (182). Oxygen saturation values differ correspondingly (75). The difference in $Po_2$ between blood from the left atrium and that in a systemic artery is about 7 mm. Hg (31). General problems on pulmonary gas exchange and its mathematical aspects have been reviewed by Loeschcke (174).

*Hemoglobin; blood gases.*—For a full and lucid exposition of the present knowledge of oxygen and carbon dioxide transport by the blood the reader is referred to Roughton's handbook article (138). Chemical and biological aspects of adult and fetal hemoglobin are presented in a monograph by Betke (26).

Specially accurate methods for oxy- and carboxy-hemoglobin dissociation in sheep blood solutions and technical developments in rapid reaction apparatus have allowed absolute values to be assigned to the equilibrium constants of the intermediate compound theory (124, 228). Several of the intermediate kinetic constants could be calculated. The velocity-constant for dissociation of an $O_2$ or CO molecule appears to be proportional to the total number of $O_2$ or CO molecules in the dissociating complex, whereas the kinetic constant for combination with a remaining vacant group is independent of the distribution of the three combined groups between $O_2$ and CO.

The dissociation curves for $O_2$ and CO hemoglobin can only approximately be made to coincide by multiplying the CO coördinate by a factor *M*. A discrepancy exists at low as well as at high (228) saturation at pH 7.1. The older concept that the velocity and equilibrium constants of the reactions of hemoglobin with $O_2$ and CO bear a constant ratio to each other has been seriously shaken.

Homogeneity of hemoglobin has been implicitly assumed in Roughton's work. In Gibson's study on worm hemoglobins it is pointed out that quite appreciable kinetic differences, attributable to the presence of more than one kind of hemoglobin, may exist without causing detectable divergences from theoretical equations (123).

*In vivo* determinations show a linear relationship between low values of alveolar CO concentration and CO hemoglobin percentage (61). In sheep the ewe's blood and that of the fetus have identical partition coefficients for $O_2$ and CO (159).

Oxygen dissociation curves, in the $P_{O_2}$ range from 0.2 to 140 atm., have been obtained from the blood of deep sea fishes at varying pH and $P_{CO_2}$. The oxygen secretion into the swimbladder cannot be explained by the Root-effect in acidified blood (234). Storage (259) and various types of anemia (158) may shift the dissociation curve.

The effect of temperature on the first dissociation constant of carbonic acid in true plasma was identical to that in separated plasma (213). The log $P_{CO_2}$ versus pH line however did depend on the presence of red blood corpus

cles and was shifted to the acid side in separated plasma at low temperature (46). In true plasma such a shift did not occur.

## Mechanics of Ventilation

*Pleural and esophageal pressures.*—The long type of esophageal balloon proved to be superior to the short one and also caused fewer artefacts than the open water-filled tube technique (190). The pressure in the lateral pleural space was found to be more negative than in the medial one. This difference varied from 3 cm. water during quiet breathing to 8 cm. at deep inspiration (71).

*Lung compliance and resistance.*—The importance of the distribution of laminar and turbulent flow in the airways has become better recognized in recent years. This distribution depends on the instantaneous flow level and on the geometrical configuration of the bronchi. Neither one is constant during a breathing cycle. The apparent constancy of linear and quadratic resistance components during breathing therefore seems to have a formal meaning only and does not denote a constant degree of turbulence or even a virtual constancy of the configuration of the airways. An analysis of the dynamic component of intrathoracic pressure and especially a determination of lung-tissue viscous resistance requires a comparison of pressure relationships during the inhalation of gases with different viscosity but equal kinematic viscosity, i.e. viscosity-density ratio (187).

Analysis of a passive expiration in anaesthetized apneic subjects has been used as a method for the determination of the compliance and resistance of the thorax and lungs (73). Even without measuring passive inflation pressure, but instead using a known added resistance, is it possible to derive the above parameters from passive expiratory flow curves (49). The fit of theoretical and experimental curves would seem to prove the validity of the underlying assumptions, viz. the constancy of laminar and turbulent resistance and elastance during the course of a passive expiration. It is difficult to reconcile the constancy of the linear and quadratic resistance constants during one expiration with their variation from breath to breath at different inflation pressures (201). This would point to a difference between elastances obtained under static and dynamic conditions. Such hysteresis effects were found to be less than one-third the static elastance (49). Reduction of lung blood volume by high inflation pressures and changes in compliance associated with changes in the functional residual air, as measured by the decompression method, may play a part in these effects (201). Hysteresis effects are also inherent in Bernstein's (21) interpretation of the pressure-volume curve of the rabbit's lung. In fluid filled lungs the bronchial smooth muscles had little direct effect on elastic recoil (222). A sudden rise in intrathoracic pressure at the end of an expiration, caused by air-trapping, was repeatedly observed not only in patients but also in normal subjects in the last fraction of the expiratory reserve (97).

It has often been suggested that, especially in pathological cases, the lungs should be regarded as a system of several compartments each with its own compliance and resistance. Mount (197) has indeed found the compliance of rat lungs, as indicated by the difference in pressures at zero gas-flow, to decrease with frequency. An arrangement in series of compliance-resistance units, containing a gas-flow resistance and a liquid-flow resistance, can be made to behave similarly to the lungs when breathing various gases. The relatively large ventilatory work per cycle at low frequencies is probably associated with tissue deformation resistance.

The chest wall cannot be regarded as passive except in deep anaesthesia or after neuromuscular blockade (49). In the conscious "relaxed" state its compliance is larger and probably assisted by inspiratory muscle tone (200). In poliomyelitis the compliance of the lungs and chest was found to be reduced in parallel with the reduction in vital capacity (65). The compensatory enlargement of the chest at the induction of a pneumothorax has already been discussed in this review (51, 52, 138, 143). The abdominal muscles have an expiratory role in the erect posture only at very high levels of ventilation (58).

*Maximal breathing capacity.*—To avoid confusion in terminology Bernstein & Kagantis (22) propose the term "maximal voluntary ventilation" (MVV), when the subject chooses freely his own breathing rate and the term "maximal ventilatory capacity" (MVC) when rate is imposed. The highest MVC is normally obtained at frequencies of 80 per min. or even higher (202). The tidal air at any rate can be predicted from fast expiratory and inspiratory vital capacity curves. The level of breathing is chosen in such a way that the optimally related portions of these curves are in use (22). The unswept fraction of the vital capacity during MVC tests is related approximately exponentially to the duration of the respiratory cycle (241). During maximal exercise the achieved ventilation is maximal for the chosen rate. Measurements of esophageal pressures have been made during the performance of the MBC test (202).

Maximal voluntary ventilation increases by 24 per cent in acclimatized men at 17,000 ft. altitude, mainly as a result of a decrease in lung gas density. The ventilatory work remains practically constant. Hypoxia, as such, causes a small but significant increase in MVV (76). Carbon dioxide, exercise, and epinephrine also increase the volume of gas that can be moved by maximal effort (169).

The work of breathing has been reviewed by Otis (205), both from the mechanical and from the metabolic point of view. The "oxygen cost" of high respiratory minute volume appears to be considerable, e.g. 800 cc./min. at a ventilation of 100 l. per min. The cost of quiet breathing amounts to from 6 to 10 cc. of oxygen per liter ventilation (238). At some intensity of ventilation a minimal alveolar $P_{CO_2}$ must exist. In cases of pulmonary emphysema this minimum may occur at low values of ventilation. A paradoxical increase of alveolar $P_{CO_2}$ has indeed been observed in voluntarily hyperventilating pa-

tients (29). Similar considerations may be applied to high altitude hyperventilation, where, in addition, the thermal cost of breathing (resulting from evaporative heat loss) brings even closer the point where winning oxygen takes all of the oxygen (185). Interesting observations and theoretical curves on the oxygen cost of breathing have been published by Cournand *et al.* (78) and Riley (226). Dyspnea does not appear to be directly related to the mechanical work of breathing, but to be conditioned, as during exercise in normals, by the force which has to be exerted on the lungs (183).

## Pulmonary Circulation

Birnbaum's monograph on the anatomy of the bronchovascular system (28), contains a 15-page atlas of human pulmonary vessels and a paragraph on experimental surgery. The pathophysiology of pulmonary hypertension has been reviewed by Harvey & Ferrer (141). Capillary microscopy has revealed interesting details of blood and plasma flow in small lung vessels (56, 57) and of the responses of terminal pulmonary artery tips to distant burns (160). Small glass spheres pass directly through pulmonary capillaries; the passage of larger spheres through anastomoses is almost negligible (39). The existence of a crossed vasomotor and bronchomotor sympathetic innervation has been convincingly demonstrated (87). Vasomotor responses in perfused lungs are not modified by shifts in acid-base balance (86).

*Pulmonary vascular resistance.*—The intraluminal pressure is an important factor in determining pulmonary vessel diameter. Resistance varies inversely with pulmonary vein pressure (133) and, in perfusion studies, with inflow pressure and flow (266). In experimental mitral stenosis the pulmonary pressure gradient initially decreases; at left atrial pressures above 40 mm. Hg, however, the gradient increases again (164), indicating an active pulmonary vasoconstriction. Halmágyi *et al.* (136) find a passive decrease in resistance in most cases of experimental mitral regurgitation and an increase, presumably of reflex-character, following experimental pulmonary stenosis (135) and tricuspidal insufficiency (137). Apparently in these cases of altered pulmonary hemodynamics both hydrodynamic and reflex phenomena are involved. Guyton *et al.* (132) measured the responses of right ventricular and other pressures to gradual and sudden occlusion of the pulmonary artery in dogs before and after elimination of circulatory reflexes. When the pulmonary artery was partially occluded the systemic blood pressure rose about 10 per cent. Apart from circulatory reflexes, the Starling effect and the coronary ischemia factor are involved in these responses. Unilateral pulmonary artery occlusion has no perceptible effect on systemic blood pressure (242) but leads to a gradual rise of respiratory rate and to unilateral ventilatory effects.

The collateral circulation which develops after ligation of the pulmonary veins of an entire lung carries at least 20 per cent of the bloodflow and causes a rise in the oxygen content of azygos blood (154, 237). Not only are preexisting channels expanded but new channels also develop after 3 to 15 days (212).

In conditions of chronically altered hemodynamics the possibility of an extensive collateral circulation between bronchial and pulmonary vessels should be considered in determining pulmonary capillary pressures and also in applying the Fick principle. Intrapulmonary oxidation and lactic acid metabolism are too small to interfere with the latter (36, 193). In perfused lung tissue obtained after venesection of dogs, however, a sevenfold increase in metabolism has been observed (38). Fluctuations of mean venous oxygen saturation can be disregarded at rest, but probably not during exercise (270). The opinions about the interpretation of pulmonary arterial and venous wedge pressures are still somewhat divergent. The capillary pressure may not be estimated by a catheter wedged in a vessel at either end of the capillary bed when the pressure gradient on the other side is not negligible (74, 119, 268).

*Responses to hypoxia.*—Pulmonary and systemic circulatory responses to various degrees of general arterial hypoxemia have been investigated by Gorlin & Lewis (128). Active as well as passive changes probably occur in the pulmonary vascular bed during such hypoxemia. Besides hypoxia, long lasting anaesthesia can produce similar changes (168). Output determinations with an isotope dilution method indicate active vasoconstriction, passive changes being taken into account. No evidence was obtained for arteriovenous shunting during hypoxia (249). An hypoxic rise in pulmonary resistance was also observed during exercise in man (240). Direct local responses were not demonstrable during unilateral hypoxia in man (112), though sometimes striking constrictory but also dilatatory effects have been observed (231). Perfusion of cat lungs with deoxygenated blood does not raise the pulmonary arterial pressure, whereas low alveolar oxygen concentrations do. The site of action of hypoxia is probably the alveolar capillary region (100).

*Lung edema and tissue fluid.*—Studies with deuterium oxide have indicated a small pulmonary extravascular tissue space (171), exchanging intensively with the pulmonary blood flow (66). Disturbances of oxidative phosphorylation processes in the capillary cells may be associated with the formation of toxic pulmonary edema (37). Respiratory movements, particularly the excursions of the diaphragm, are of paramount importance for the lymph drainage of the serous cavities and lungs, a subject reviewed by Courtice & Simmonds (79).

## General

*Upper and lower airways.*—The normal and pathological physiology of nasal and oral respiration has been discussed by Eckert-Möbius (101). The air temperature along the respiratory tract has been carefully measured at various environmental conditions (70). The related problem of evaporative water loss by respiration under various environmental conditions has been studied by Thauer *et al.* (254, 255). Determinations of the pH of the bronchial mucosa and of a fluid layer exposed to the bronchial air can be used as a method for local intrabronchial $CO_2$-analysis (245). The rate of ciliary beat

and mucous flow in the trachea were found to be about 1400 and 14 mm. per min. respectively (85). Acetylcholine acts as a local hormone for ciliary movement (55). Electron microscopic studies have revealed new structural details of cilia (108, 217). The degree of stagnation of bronchial secretion has been found to be directly related to the extent of experimental bronchiectasis in experiments with complete, partial or temporary occlusion of rat bronchi (64).

*Alveoli.*—Only a few of many electron-microscopic studies can be cited (69, 216). Prolonged fixation with osmic acid may dissolve endothelial and epithelial cytoplasm (177). The mitotic activity of alveolar cells (25) and the production of alveolar surface fluid (179) are probably of importance with respect to the removal of dust from the lungs (215).

*Circulatory effects of ventilation; artificial ventilation.*—Venous return is immediately increased upon the onset of inspiration and brings about an enlargement of the volume of the next stroke (42). The effects of the Valsalva maneuver have been studied in detail in normal subjects (130, 166) and in cases of pulmonary congestion (32, 189). The negative phase in positive-negative pressure respiration does not benefit the circulation in an open chest preparation (8). In closed chest preparations its advantage over positive pressure respiration is especially apparent in hypovolemic conditions (153). Negative pressure breathing increases urine flow without affecting sodium excretion (251). A report has been issued on gases administered in artificial ventilation (95) stressing the dangers of carbon dioxide. Ventilation standards for use during prolonged artificial respiration in patients have been presented in nomographical form (221). The subject of modern automatic respirators and respiration techniques is not reviewed.

*Abnormal gas pressures.*—A report on compressed-air illness in tunnel workers containing information on new recompression methods has appeared (210). Woolmer has given an account of problems of underwater breathing at various depths (271). Further studies have appeared on the anaesthetic action of inert gas (62), neurogenic and adrenal medullary and cortical factors in oxygen poisoning (18, 121), and on the pathogenesis of retrolental fibroplasia (45).

*Clinical physiology; books and reviews.*—An extensive survey of clinical physiologic literature has been presented by Altschule (6). Comroe *et al.* (72) have written an outstanding book on pulmonary function tests which is especially useful for clinicians. Drinker's monograph (98) deals mainly with problems of blood- and lymph-circulation in the lungs. Many aspects of clinical respiratory physiology have been covered in a symposium (104) and in reviews (118, 196). The hematologic rôle of the lung in man has been reviewed by Bierman (27). References to other books and reviews will be found in the foregoing sections of this review. Special mention should be made of the Handbook of Respiratory Physiology (138), edited by the late Dr. W. M. Boothby.

*Techniques.*—Physiological requirements of oxygen systems for high

altitude have been pointed out by Roxburgh (229). The use of oxygen equipment (138) and various methods for measuring respiratory flow, gas concentration, and exchange (60) are discussed by Carlson. An integrating, low inertia, motor pneumotachograph (114) and a ballistic apparatus measuring the impulse of forced expiration (181) have been described. Wildhack (265) has reviewed principles of fluid and gas flow measurement. The correction for "effusion velocity" in the volume-expansion method for measuring residual capacity has been studied by Harvay & Schilling (140). Descriptions have been given of a closed-chamber method for recording ventilation in small animals (63), a body plethysmograph (34), an infrared $CO_2$-analyzer (50), a new nitrogen meter (178), and manometric (150) and mass-spectrometric (211) methods for blood gas analysis in the presence of volatile anaesthetic agents.

Photometric methods for estimating oxygen saturation in small blood samples (195, 244) and for continuous ear-lobe oximetry (236, 247), as well as methodological studies in this field (2, 9, 102, 167, 191, 263) have been published. The applicability of various hemolytic agents in the Scholander-Roughton method has been tested (155). Odorants accelerate saponin hemolysis (90). A more rigid procedure for Riley's bubble method has been proposed (47). A multiple platinum electrode has been used for recording oxygen tension in flowing whole blood (194). Burk & Hobby (54) point out several interesting hydraulic principles for magnification of sensitivity in free and fixed volume manometry. Holaday has published a detailed method for serial determination of pH in arterial blood (149). Methods for direct puncture of the left heart and the great arteries have been described (5, 30, 35, 223). Many other techniques have been dealt with earlier in this review.

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# DIGESTION[1]

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## Salivary Secretion

Thaysen, Thorn & Schwartz (1) studied the concentrations of sodium, potassium, chloride, and carbon dioxide in human parotid saliva, secreted at various rates in response to methacholine (Mecholyl). The concentration of sodium increased with rising secretory rates, but was always lower than in the plasma. Conversely, the concentration of potassium was largely independent of the secretory rate and higher than in the plasma. The authors conclude that both ions are delivered into a precursor solution in amounts which are simply proportional to the rate of salivary flow. Sodium, but not potassium, is in part reabsorbed from this precursor solution by a subsequent process of limited capacity The rate of secretion of the various salivary glands was determined by Schneyer & Levin (2). The submaxillary glands were found to contribute approximately 69 per cent, the parotid glands 26 per cent, and the sublingual glands 5 per cent of the total secretion from these major gland pairs.

The mean salivary concentration of sodium, potassium, and chloride in 73 normal subjects in the fasting state after chewing paraffin was found by White *et al.* (3) to be $Na = 26.4 \pm 11.8$, $K = 19.7 \pm 39$, and $Cl = 29.0 \pm 8.8$ mEq/L. It was suggested that salivary sodium or chloride concentrations or both are hypotonic to their respective serum concentrations, as a result of reabsorption of these ions by rodded epithelium in the excretory ducts. Potassium is believed to be actively secreted, since its concentration in the saliva is higher than in the serum.

Using the parotid gland of the cat, Strömblad (4) has supplemented the observations of Emmelin & Muren on the submaxillary gland i.e., that sympathetic denervation renders the gland more sensitive to the secretory action of acetylcholine, epinephrine, and pilocarpine. Cannon's "law of denervation", a greater sensitization after postganglionic than after preganglionic denervation, was found to be valid for acetylcholine and pilocarpine. The reverse seemed to apply to epinephrine. Some evidence was given to show a dual action of sympathetic stimulation and of epinephrine, one secretory and one excretory.

## Food Intake, Appetite, Hunger, Thirst

The correlation between food intake, body weight, and exercise was studied in rats by Mayer *et al.* (5). Slight exercise in a treadmill did not influence the food intake, and the body weight decreased. More strenuous

[1] The survey of literature pertaining to this review was completed in April, 1955.

exercise increased the food intake, and the body weight remained unaltered. Very hard exercise reduced both body weight and food intake, the latter presumably resulting from fatigue. The authors stress the importance of considering exercise as well as food intake in the treatment of obesity. Sellers, You & Moffat (6) exposed rats to a cold environment (1.5°C.) and fed them on diets with a constant protein content but a variable fat content (5 to 44 per cent). The caloric intake was remarkably constant regardless of the amount of lipide present in the diet. Thus, a high fat content led to a decreased consumption of protein. No significant difference with regard to survival rate or to increase in body weight occurred, which was attributable to the amount of lipide present in the diet. Pyrogenic reactions after intravenous injection of amino acid solutions were observed by Beamer & Thomas (7) to be accompanied by a decreased food intake. The pyrogens were assumed to influence the appetite via the hypothalamus.

Anand, Dua & Shoenberg (8) produced aphagia by bilateral electrolytic destruction of the lateral hypothalamus, and hyperphagia by similar destruction of the ventromedial nucleus in cats and monkeys. The authors agree with the hypothesis that a "satiety centre" in the lateral hypothalamus and a "feeding centre" in the medial hypothalamus facilitate and inhibit primary feeding reflexes initiated by optic, acoustic, gustatory, olfactory, and mechano-receptors. Slowly-adapting stretch receptors were found in the stomach of anaesthetized cats by Paintal (9). Most of them were localized to the pyloric portion. The receptors behaved similarly to other stretch receptors in skeletal muscles and the lung. The author made an interesting suggestion, namely, that the function of the receptors is to constitute the peripheral mechanism for the immediate satiation of hunger and thirst. Gastric stretch receptors and other receptors in the stomach and intestines responded to phenyl diguanide, 5-hydroxytryptamine, nicotine, and other substances (10).

Richter & Mosier (11) found that rats are able to handle very large amounts of salt, 15 to 20 gm./kg. of body weight per day, provided that they are allowed to ingest sufficient quantities of water. Domesticated rats need 50 to 60 cc. of water/gm. of salt to maintain health. Ingestion of large amounts of salt over 2 to 3 months did not lead to any visible dietary deficiencies, nor did it produce any detectable pathologic changes in the kidneys. When given access to water, rats will freely eat diets containing as much as 50 to 70 per cent salt. Di Salvo (12) confirmed and supplemented previous observations that, above a certain threshold value, the drinking response of the dog to the intravenous injection of NaCl is proportional to the total amount of salt given but independent of the concentration of the salt solution. The drinking response can be completely inhibited by preceding the salt injection by intravenous injection of water in volumes slightly in excess of the volumes normally ingested after such doses of hypertonic salt solution (13).

## Gastric Secretion

The role of the gastric antrum in the regulation of HCl secretion has been studied in several laboratories. Woodward *et al.* (14) have confirmed the observations of various earlier workers that distention of an antrum pouch, and its perfusion with macerated liver or protein hydrolysates, produces HCl secretion in a Pavlov pouch or a total stomach pouch. They also reinvestigated the effect of instillation of cocaine (2.5 per cent) and atropine (1 per cent) solutions. They were found to block the secretory response to mechanical (but not in all experiments) and to chemical stimulation of the antrum mucosa. The observations afford further evidence of the correctness and significance of Edkins' gastrin theory.

Of great interest is the report that instillation of 0.1 *N* HCl into the antrum pouch inhibited the spontaneous secretion observed in one dog, and that distinctly acidified liver homogenates evoked a smaller secretory response, or none. The secretion produced by histamine, or occurring in a Heidenhain pouch 1.5 to 2 hr. after a meal, was not inhibited by instillation of HCl into the antrum, nor was the secretion induced in a Pavlov pouch by hypoglycaemia. The introduction of HCl into the duodenum was not found to inhibit the secretory response in a Pavlov pouch to a meal or to insulin. Woodward *et al.* conclude that the inhibitory effect of HCl (and of other $H^+$ ions) is attributable to interference with gastrin formation or liberation, and not to the appearance of any inhibitory substance from the antrum or duodenum.

The interdependence between the gastrin mechanism and the nervous phase of gastric secretion is still debated. Linde (15) evoked in cats a constant gastric secretion with low peptic activity, but HCl secretion increased only after a latency of 15 to 25 min. Instillation of acetylcholine into a pyloric pouch elicited a secretion of HCl, but not of pepsin, in the fundus. Linde concludes that the acid and the pepsin secretion during vagal stimulation are initiated by different mechanisms. The acid secretion is assumed to be stimulated by a neuro-hormonal mechanism involving the acetylcholine-gastrin-histamine chain, whereas the secretion of pepsin is stimulated directly by acetylcholine liberated by the vagus nerves.

Pevsner & Grossman (16) succeeded in evoking HCl secretion by the infusion of small amounts of acetylcholine into the arteries of the gastric fundus of the dog; larger amounts caused a suppression of acid secretion. Infusion of acetylcholine into the arteries of the pyloric portion of the stomach induced HCl secretion with both low and high acetylcholine concentrations. A copious secretory response to insulin (0.5 units per kg.) was observed after extirpation of the antrum and the entire small intestine, thereby removing all known sites of gastrin formation. It was concluded that, in the dog, vagal stimulation of gastric acid secretion is mediated mainly by direct cholinergic action on the fundal glands. The gastrin release by vagal impulses is considered to be a minor and dispensable auxiliary

mechanism. The loss of possible inhibitory mechanisms by removing the duodenum is not discussed by the authors.

The intricate question of the role of the duodenum in control of gastric secretion was reinvestigated by Brackney, Thal & Wangensteen (17). They showed in Heidenhain dogs that the HCl response to a test meal increased from 84 to 820 per cent by resection of the duodenum, or by transplanting it further down into the jejunum. Their observations strongly support the earlier concept of an inhibitory mechanism localized to the duodenum. Varró *et al.* (18) observed a latency between the secretory response to insulin hypoglycaemia of the HCl and the peptic cells. The latency disappeared after antrum resection, and was ascribed to the removal of a hormonal mechanism. Gastric secretion was induced in recipient dogs by injection of blood (2×40 cc.) from hypoglycaemic dogs. Since no hypoglycaemia occurred in the recipient dog, the secretory response in this animal was ascribed to the effect of a secretory substance (gastrin?) circulating in the donor animal.

Walker, Olson & Necheles (19) conclude from observations on dogs with denervated total stomach pouches that the fasting normal dog probably has no fasting secretion. Secretion of HCl which occurred was ascribed to gastrin liberation in the antrum resulting from the reflux of duodenal content or other stimuli. Hill (20) reached a similar conclusion as regards the ruminants. It is true that a continuous secretion of gastric juice occurs in the stomach of fasting sheep and goats, but ingesta can be collected from an abomasal fistula of such animals during starvation periods up to 48 hr., and the secretion of gastric juice ceases if ingesta are prevented from entering the abomasum by emptying the fore-stomachs.

The intragastric pH during a meal was found by Henning, Kinzlmeier & Kimbel (21) to be between 3 and 5. The widespread use of the Shay rat method in screening antiulcer drugs led Shay, Sun & Gruenstein (22) to work out a quantitative method for measuring spontaneous gastric secretion in the rat. The gastric juice secreted during a 4-hr. period after pyloric ligation is measured, and by using sufficiently large groups of animals (at least 10) statistically significant results are reported. Surprisingly high acidity values were obtained by Atkinson & Henley (23) in samples collected simultaneously from the stomach and the first part of the duodenum. In control patients, the pH did not exceed 3 during a 24-hr. period, the acidity being higher in the stomach than in the duodenum. In duodenal ulcer patients, the gastric and duodenal acidity was higher; in gastric ulcer patients it was lower than in the controls. In the latter the intragastric and intraduodenal acidity fell towards neutrality during the night, indicating that acid might play a different role in the pathogenesis of gastric and of duodenal ulcer.

*Inhibition.*—Davenport, Chavré & Davenport (24) inhibited the HCl secretion of isolated gastric mucosa from the mouse and frog produced by Carbachol (carbaminoylcholine) with *p*-chloromercuribenzoate. They concluded that the inhibition was attributable to blockade of two sulfhydryl

groups occurring in a substance necessary for the production of HCl, but of unknown nature and not identical with previously known substances obligatory to oxidative processes.

Texter & Barborka (25) were able to produce significant inhibition of HCl secretion in the human stomach by giving 1 gm. per 24 hr. of acetazoleamide (Diamox), the new, powerful carbonic anhydrase inhibitor. They consider their observations to support the view that carbonic anhydrase in the parietal cell plays an important role in the process of HCl secretion of the stomach. As is known, the father of the carbonic anhydrase theory, Davenport, has become less certain regarding the role of this enzyme in HCl production.

Urine extracts from hypophysectomized dogs, in small doses (1 mg.,) produced an increase, in larger doses (3 mg. or more), a delayed inhibition, of the secretory response to histamine in Heidenhain pouch and gastric fistula dogs [Kaulbersz, Patterson & Sandweiss (26)]. The absence of the typical prompt inhibition, characteristic of normal urogastrone, was taken to indicate some effect of the hypophysis on urogastrone production.

Intravenous administration of a pyrogen, a bacterial polysaccharide, depressed the histamine-stimulated HCl secretion in total stomach pouches, even when the pyretic reactions were depressed by a salicylic preparation such as acetylsalicylic acid [Olson, Walker & Necheles (27)].

The inhibitory action of insulin hypoglycaemia on the histamine-induced gastric secretion in Heidenhain pouches of dogs was shown by Forrest & Code (28) to be uninfluenced by sympathectomy; it was therefore supposed to be related to the decreased concentration of sugar in the blood.

It has been demonstrated previously that ethionine, the synthetic ethyl analogue of methionine, causes destruction of the acinar cells of the pancreas, with consequent impairment of pancreatic secretion. No demonstrable change was observed by Kroboth & Hallenbeck (29) in the secretion of pepsin or HCl in Heidenhain pouch dogs given 1 to 25 mg./kg. body weight of ethionine for 3 to 23 weeks. Gastric secretion was stimulated by feeding or histamine. Forrest & Code (30) ascribe the reduction of the secretory response in a Heidenhain pouch to a meal to accelerated emptying of the stomach. Van Noordwijk & Aarsen (31) observed in Shay rats that histamine- and pilocarpine-induced secretion of HCl was inhibited by tetrabromo-tetrachlorofluorescein (Phloxin). Since Phloxin precipitates histamine, the observations are taken to indicate that histamine is the normal humoral transmitter in the stimulation of the HCl glands.

*Kinetic factors.*—Adam *et al.* (32) studied the dose-response curve for the effect of continuous intravenous infusion of histamine on the HCl secretion in man. The validity of their conclusions that the secretory maximum is the most important parameter for defining the secretory response to histamine, and that the free histamine in the blood is one reason for the basal HCl secretion, seems questionable. Gastric secretion was evoked in cats by continuous intravenous injection of histamine. Various portions of the plasma chloride

were replaced by bromide, either by adding NaBr to the food or by intravenous injection. The difference observed between the relative output rates of Br and Cl was considered to be inconsistent with the theory that the halogens penetrate the mucosa by simple diffusion, but to support the concept of the halogens passing the mucosal membrane in a combined state [Heinz, Öbrink & Ulfendahl (33)]. Studies on isolated frog mucosa by Hogben (34) also support the view that chloride is actively transported through the gastric epithelium, thus providing the ionic current generating the spontaneous mucosal potential. It is supposed that the chloride ion moves through the mucosa in an undissociated state and hence implies a carrier. Whether chloride transport is localized to surface mucous or parietal cells is obscure.

Parenteral administration of ascorbic acid to guinea-pigs with gastric fistulas was reported by Breidenbach & Ray (35) to increase the normality but not the rate of the secretion of the gastric juice. This is a rather surprising observation since, according to current opinion, HCl is secreted with a constant normality. Mizell (36) confirms the current view that gastric secretion in frogs (*Rana pipiens*) undergoes seasonal variations, but shows them to be independent of the temperature at which the animal lives (28°C. and 4°C.). The cycle shows maximal decreases in pH in late spring and late fall, although all seasons differ significantly from each other.

*Influence of endocrine factors.*—McCarthy, Evans & Dragstedt (37) observed profound and continued hypersecretion in Pavlov and Heidenhain pouches of lactating dogs. Pregnancy had no consistent effect on gastric secretion, nor had prolactin. Sun *et al.* (38) observed in rats that athyroidism and hypothyroidism cause a depression in the rate of secretion and total acid output of the gastric secretion. The thyroid gland is supposed to influence gastric acid and pepsin secretion through the adrenocortical pathway. Despite a raised plasma histaminase level, no changes were observed in the spontaneous or histamine-induced gastric secretion in nine women during pregnancy or after parturition [Clark & Tankel (39)]. Hypophysectomy in rats was found to cause severe involution of the gastric zymogenic cells and a reduction in the secretion of pepsin, as early as three days after operation [Baker & Abrams (40)]. From further studies on hypophysectomized rats, Abrams & Baker (41) conclude that the anterior hypophysis may regulate zymogenic cells by way of the thyroid and adrenal cortex, the latter gland being primarily involved. Post-histamine plasma pepsinogen was recorded in normal and thiouracil-treated guinea-pigs by Kowalevski *et al.* (42).

## Peptic Ulcer

Dragstedt *et al.* (43) showed that prolonged contact of the gastric antrum with food, and excessive peristalsis and distention of the antrum resulting from pyloric stenosis, cause hyperfunction of the antrum with hypersecretion and peptic ulcer formation in dogs. They state that these factors are also

present in many patients with peptic ulcer. Duodenal ulcers, on the other hand, are ascribed to a hypersecretion of gastric juice of nervous origin, which is stated not to be abolished by gastrectomy or antrum resection.

In contrast to previous reports on the enhanced HCl secretion and increased ulcer frequency after administration of ACTH and cortisone, Sandweiss *et al.* (44) observed that ACTH treatment prolonged the survival time of Mann-Williamson dogs and reduced the incidence of ulcer. Cortisone treatment prolonged survival time but did not reduce the incidence of ulcer. Sandweiss *et al.* (45) reported that oral treatment with an extract from pregnant mares' urine lowered the ulcer incidence and lengthened the survival time in Mann-Williamson dogs. Lüthi (46) produced peptic ulcers in guinea-pigs by injection of anterior hypophysis extracts, and Kowalevski & Bain (47) prevented histamine-induced ulcers in these animals by pretreatment with extracts of the posterior hypophysis (Infundin, Burroughs Wellcome). Detrick *et al.* (48) studied the effect of x-irradiation of the gastric secretion and the gross and histologic changes in the Shay rat stomach. Steigmann & Dolehide (49) observed a greater increase in gastric acidity and a more marked decrease in antral motility after smoking an ordinary rather than a filtered cigarette. Since Brummer (50) observed ulcer pain after histamine-induced gastric secretion only in a few ulcer patients, he doubts whether ulcer pain is attributable to the effect of the HCl.

## Emesis

According to Wang & Glaviano (51), Hydergine and morphine produce emesis by stimulating the emetic trigger zone of Wang & Borison. The zone is also essential for the production of motion sickness in dogs [Wang & Chinn (52)].

Since injection even of large doses of Hydergine, apomorphine, and lanatoside C failed to produce emesis in the monkey, Brizzle *et al.* (53) concluded that the chemoreceptor trigger zone is virtually nonfunctional in this animal from the standpoint of drug action. Exposure to x-irradiation resulted in emesis, which could be prevented by ablation of the trigger zone.

## Pancreas

The role of the pancreas in the regulation of lipide metabolism is still obscure. Dragstedt *et al.* (54, 55) produced in dogs fatty infiltration of the liver, a decrease in the blood lipide level, and other well-known metabolic disturbances due to depancreatization, to ligation, and to secondary destruction of pancreatic tissue. The deficiency syndrome was completely prevented or relieved not only by feeding raw pancreas, but also by feeding autoclaved pancreas, whereby enzymes must have been destroyed. Subtotal pancreatectomy, leaving only a remnant weighing 1 to 2 gm. attached to the pancreatic duct, resulted in diabetes (which could be controlled by insulin treatment) and in hyperlipaemia. Deviation of the pancreatic juice from this small remnant to the exterior lowered the insulin requirement and

abolished the hyperlipaemia. Diabetes reappeared on ligation of the pancreatic duct with destruction of the pancreatic remnant. If such dogs were fed fresh beef pancreas, hyperlipaemia occurred. The authors suggest that the pancreas influences lipide metabolism by means of internal secretion of a substance (lipocaic) which potentiates the lipotrophic effect of choline and similar substances in the diet. In the absence of pancreas, or when endocrine function is impaired, the presence of pancreatic juice in the intestines causes hyperlipaemia and a high insulin requirement. The withdrawal of pancreatic juice under the same conditions causes hypolipaemia, decreased insulin requirement and tolerance, and fatty infiltration of the liver. On the other hand, Sachdev (56) points out the close relationship between proteolytic enzymes and the pancreatic antifatty liver action by feeding trypsin, chymotrypsin, and papain to rats with the pancreatic duct ligated.

Lin & Grossman (57) stimulated the amylase secretion from the pancreas by the continuous administration of urocholine and secretin. The simultaneous or previous injection of DL-methionine prevented the depressive action on the enzyme secretion of DL-ethionine. The stimulating effect of histamine on the pancreatic secretion in man was considered by Dreiling (58) to be secondary to the secretion of HCl. The precise technique of the secretin test and normal ranges for the volume, bicarbonate, and amylase secretion from data of 123 patients without pancreatitis, studied with secretin (Lilly) were presented by the same author (59). Histochemical examination of the pancreas of the dog, sheep, goat, horse, rabbit, and cat revealed that only in the dog did the cytoplasm of the gland cells, both acinar and islet cells, contain pseudocholinesterase. In this animal the enzyme was also identified in the pancreatic juice. It is suggested by Hebb & Hill (60) that the cholinesterase has a catalytic role within the cell and is not itself a secretory product of any significance, being secreted from the alveolar cells after fulfilling its role there. Recording the pH in the pancreatic tissue of narcotized rabbits with a gold-iridium-hydrogen electrode, Held & Maske (61) found values around $7.98 \pm 0.38$, which are significantly higher than in skeletal muscles ($7.03 \pm 0.38$). Negative effects of long-term treatment of rats with glucagon were reported by Galansino *et al.* (62).

## Bile

In rats with the bile duct cannulated, the biliary excretion of cholesterol was found by Thompson & Vars (63) to be increased in hyperthyroidism and decreased in hypothyroidism. Cholic acid excretion was lowered in the former condition and was normal in the latter. Among factors affecting bile flow in the rabbit and rat, Danforth & Dummit (64) claim inhibition by large doses of epinephrine, hypoxic breathing, and low temperature. Bile acids given orally to patients or painted directly on the intestinal mucosa of an intestinal fistula produce increased motor acitivity [Bergman (65)]. Since scopolamine-*N*-butylbromide (Buscopan) prevented the motor effect of the bile acids, the author considers it to result from a peristaltic reflex originating in the mucosa. Brauer, Leong & Holloway (66) perfused isolated rat livers

and determined the relations between perfusion temperature, rate of bile production, and maximal bile secretion pressures attained.

## Intestines

*Motility.*—The role of acetylcholine in intestinal motility is discussed by Goffart (67) and Goffart & Bacq (68). The acetylcholine content of the intestinal canal was observed to be uninfluenced by total denervation, the highest values being found in the jejunum. In eserinized dogs, the venous blood from the stomach, jejunum, ileum, and colon contained acetylcholine. This substance was released from denervated intestines and during postoperative atonia, and was not, therefore, considered to result from the lack of acetylcholine. Duncan (69) claims that the inhibitory effect on the gastric musculature of the sheep produced by injection of acetylcholine is attributable to liberation of epinephrine; consequently acetylcholine should be the chemical mediator of the vagus in ruminants as well. McDougal & West (70) inhibited the peristaltic reflex and reduced the nicotine-produced contraction of the longitudinal muscle of the guinea-pig ileum with epinephrine, norepinephrine, and other dihydroxyphenylalkylamines. The action is ascribed to a depression of the transmission in the ganglionic synapses within the enteric plexus. The electrical activity was recorded by Milton, Smith & Armstrong (71) in subcutaneously localized duodenal loops in dogs. Two activity components were found: slow rhythmic waves and, if visible movements were present, series of rapid deflections. The slow waves were uninfluenced by drugs, e.g., atropine, neostigmine, and epinephrine, which abolished or augmented the visible motor activity and the rapid deflections. The slow wave activity was, therefore, suggested to be associated with the mechanisms which stimulate and coordinate the contractions of the duodenum, possibly the nervous networks of the intestinal wall.

Lorber & Shay (72) criticize the commonly-used balloon technique for recording gastrointestinal pressure, and recommend the use of open-lumen tubes connected to electrical transducers, and recorded with strain-gauge amplifiers. An *in vivo* method for studying intestinal motility in the unanaesthetized rabbit is described by Farrar, Small & Ingelfinger (73). After a midline abdominal incision, the cranial ends of two polyethylene tubes were passed subcutaneously to the back of the neck, out through a skin incision and fastened there. The caudal ends were passed down 15 and 25 cm., respectively, into the intestine through a stab wound in the stomach. The intestinal motor activity and the responses to mechanical and pharmacological stimuli could be studied in the same animal for several months. Blankenhorn, Hirsch & Ahrens (74) describe transintestinal intubation with polyvinyl tubing, by which reproducible measurements of the length of the human intestinal tract and repeated sampling of the intestinal content can be made. Rosenblum & Cummins (75) observed a definite reduction in motility of the sigmoid colon during naturally or sodium amobarbital-induced drowsiness and sleep.

Kuru & Hukaya (76) recorded the motor response of the bladder and

rectum of narcotized decerebellated dogs to medullary stimulation. The distribution of points at which stimulation produced contractions of bladder and rectum was especially dense in the grey matter adjacent to the solitary fascicle, just caudal to the obex. Points yielding relaxation of these organs were especially confined to the reticular formation ventral or ventrolateral to the solitary fascicle at the middle of the ala cinerea. The results were taken to indicate the localization of medullary autonomic centres of importance for reflex micturition and defaecation.

*Absorption.*—The effect of various factors on the intestinal absorption of physiologic saline was investigated by Blickenstaff (77, 78, 79). Eleven experiments on two unanaesthetized dogs with jejunal ring fistulas showed that the rate of water absorption in the jejunum from an isosmotic NaCl solution was reduced after a previous distilled water absorption period. When 2 per cent meralluride, or 0.003 m*M* mercuric chloride was added to 0.9 per cent NaCl solution, the absorption of sodium, chloride, and water in jejunal fistulas was retarded. On the other hand, vasopressin (Pitressin) injected subcutaneously increased the water absorption from isosmotic saline. The similarity in behaviour between the intestinal and renal tubular epithelium is pointed out. In rats with ileum and colon fistulas containing sodium chloride of various concentrations, the absorption of sodium and chloride ions occurred against a concentration gradient. The amounts of both ions absorbed increased linearly with the concentration until a concentration higher than 1.25 per cent NaCl was reached [Budolfsen (80)]. The absorption of water from the small intestine was observed by Lee (81) to cause intravascular haemolysis. The instillation of water into the intestine of pentobarbital-anaesthetized rats was followed by a rise in the hemoglobin concentration of the portal plasma, and haemoglobinuria. Lavage of the small intestine of cats with warm water (37°C.), and of dogs with cold water (15°C.) was also able to produce an increase in plasma hemoglobin.

Blomstrand (82) reports from studies on rats with thoracic duct fistulas that the major part of the absorbed labelled linoleic acid was recovered in the lymph neutral fat, regardless of the form in which it was fed. He concludes that there are no differences in the route of absorption of saturated and unsaturated long-chain fatty acids. Determining the digestibility of various glycerol esters of stearic acid, Scribante & Favarger (83) found the monostearate to be the most digestible. Magee (84) confirms the observation that the faecal fat excretion in dogs and rats decreases on protein feeding. This fact is considered probably not to be attributable to changes in bile acid output. Studying the absorption of amino acids in sacs of everted small intestine of hamsters, Wiseman (85) states that the mechanism of active transport (against a concentration gradient) is limited to the mono-amino monocarboxylic acids, and that these acids compete with each other for this mechanism.

Whether cholesterol is absorbed in the free or esterified form has been discussed for several years. Swell *et al.* (86) conclude from feeding experi-

ments on rats and *in vitro* observations that dietary fats serve a dual purpose. They stimulate the flow of bile necessary for cholesterol esterase activity and provide the fatty acid necessary for cholesterol esterification. However, cholesterol was absorbed even when the animals were fed a fat-free diet, provided that bile salts were given, a fact indicating that fatty acids necessary for esterification can originate from endogenous sources. On the other hand, Swell *et al.* (87) observed a greater rise in blood cholesterol in rats when they were fed free cholesterol or cholesterol butyrate than when fed less easily split oleate and linoleate esters. The authors considered it warranted to conclude from the latter observations that cholesterol must be in a free form before it can be absorbed. Evidently, more experiments are needed to clarify the process of cholesterol absorption.

In experiments on rats, toluidine blue was observed by Klein *et al.* (88) to be absorbed from the duodenum, and when injected intravenously to pass through the mucosa into the duodenal lumen.

### Miscellaneous

The effect of total gastrectomy on the absorption of carbohydrate, fat, and protein was studied in dogs and human subjects by McCorkle & Harper (89) and by Johnson, McCorkle & Harper (90). They found it possible to maintain satisfactory nutrition in both species if appetite and capacity for food and digestive comfort could be achieved. Scholer & Code (91) report a surprisingly high rate of absorption of heavy water from the stomach of 15 healthy persons. It is to be hoped that the water actually remained in the stomach, and was not emptied into the duodenum during the time of observation. Gastric emptying and food intake in mice were found by Fenton & Dickson (92) to be reduced for a period of less than 24 hr. after irradiation with 500 r. The "dumping syndrome" was produced by intrajejunal administration of hypertonic solutions or starch by Roberts *et al.* (93). A shift of plasma water into the intestinal lumen with an acute decrease in circulating blood volume resulted. Subsequent stimulation of the pressor receptors was suggested to constitute part of the sympathetic component of the "dumping syndrome."

The effect of epinephrine and norepinephrine on duodenal secretion was studied by Hartiala & Toivonen (94) on dogs with duodenal pouches. From 0.025 to 0.040 mg. of epinephrine per kg. of body weight increased the secretion in half of the experiments, but norepinephrine did so in only 3 out of 17 experiments. Toh (95) claims that in dogs the portal blood contains a greater amount of 5-hydroxytryptamine (serotonine) than arterial blood, and that the perfused stomach continuously releases this substance. Hwang (96) has continued his thorough studies on the mechanism of transportation of the content of the eosophagus. The sheep's rumen contains volatile fatty acids (e.g., isobutyric, isovaleric, and 2-methyl-butyric acid) (97) and reduces sulfate to sulfide (98).

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# PERIPHERAL CIRCULATION[1]

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It was intended that the present article should deal with the complete literature of the field, published from June 1954 to June 1955, but unfortunately it does not do so. Thus, papers appearing in journals which have been delayed in delivery could not be included, and with all probability others have simply been overlooked, for which apologies are extended.

There seem to be as many definitions of peripheral circulation as there are workers in the field. The definition here preferred covers a discussion of all papers dealing with physiological aspects of the circulatory system with exclusion of those with bearing on the heart and its performance, which are dealt with elsewhere in this book. Pharmacological and therapeutic studies are not discussed here, interesting as they may be. Further, to avoid overlapping, brief preliminary reports are generally omitted, as the studies can and will be dealt with more satisfactorily in future reviews when they have been published in full detail.

## Apparatus and Methods

*Pressure and pulse wave measurements.*—New manometers, suitable for measuring pressures (190) or pressure differences (or both) (37, 333) have been described, with one of which (333) the author has very favourable personal experience. In man the left atrium has been punctured percutaneously (43, 355) allowing a measurement of the pressures in this and adjacent parts of the cardiovascular system. Also the pulmonary artery can be punctured for pressure measurements, either percutaneously (356) or by endoscopy from the main bronchus (141). This may be of great advantage as it is evident from another study (457) that the movements of the usual catheters, caused by the beating of the heart, can produce significant artifacts in the recorded pressure curves. Further, a simple and convenient procedure for arterial catheterisation, useful both for obtaining samples and pressure records, has been worked out (34). Schroeder's method for pressure measurement has been compared with a direct recording of arterial pressure (176), and at more pronounced pressure changes the error is rather big. Some other more or less simple techniques for continuous recording of pressure variations (17, 53, 204) and mean pressure (25) are described and also methods for evaluation of the pressure in smaller arterial vessels (52, 277). The techniques for indirect measurement of arterial pressure in rats has been critically analyzed, and a new modification of satisfactory exactness is described (351). On the whole, in the last ten years the enthusiasm for new, more or less intricate ways to measure arterial blood pressure has been so great that one

[1] The following abbreviations have been used in this chapter: VDM (Vasopressor material); VEM (Vasoexcitor material); ATP (adenosinetriphosphate).

sometimes feel inclined to make a reminder that the old mercury manometer, writing on a smoked drum, is for a variety of purposes still a cheap, secure, and simple way to get the mean bood pressure.

A new sphygmotonograph, which may be useful in clinical work, has been constructed and tested (96), and a technique for bilateral oscillography has been described (233). Lastly the performance of the "Syncardon" apparatus has been studied under a variety of conditions (449).

*Blood flow measurements.*—A modification of the Grant-Pearson plethysmograph (226) and a plethysmograph suitable for the dog's hind limb (241) are described, and Burch (61) presents his method for finger plethysmography, which, among other things, allows a fair analysis of the phasic inflow and outflow of blood. As could be expected impedance plethysmography has been found inadequate for accurate estimations of the blood flow (30). A comparison has been made between a direct recording of muscular blood flow and the radioactive sodium chloride clearance method (350), and it was found that the clearance method can hardly be utilized as a reliable indicator of the blood flow under all circumstances, though sometimes it evidently may be fairly satisfactory (438).

Two modifications of the Gibb's method for estimation of regional blood flow changes in the brain (292) and the muscles (221) have been tested and found satisfactory, at least for some purposes. For recording temperature changes, caused by variations of the blood flow, the copper-tellurite-copper thermocouple is very suitable, thanks to its superior sensitivity (235). The use of the Pitot tube principle adopted for estimation of flow velocity has been discussed in some detail (319), and a modification of the Shipley-Wilson rotameter has been constructed (29). An attempt to use the electromagnetic flow meter principle to measure the limb blood flow in intact man was naturally enough less successful (325), while Green's latest model of the square wave electromagnetic flow meter shows a surprisingly good performance with an almost straight line correlation to the actual mean volume flow through the unopened vessel (109). The drop recorder, operating an ordinate writer, a method much used in some laboratories, has been modified to record also arterial inflow (288). The drawback that transient errors in flow measurement are obtained when the blood pressure simultaneously changes can be minimized by making the volume of the air chamber so small that it corresponds to the volume of only 8 to 10 drops, a modification that the author has utilized with satisfactory results.

For those interested in flow velocity measurements an easy way to follow the fluorescein content in the blood is described, which especially in clinical work may simplify the procedures (50). A device for measuring relative blood viscosity has been constructed (425) as has another apparatus for direct observation of some hemodynamic events in capillary tubes (427). The Jerrard-Burton apparatus, constructed mainly for demonstrations and teaching of hemodynamics, is found to be very satisfactory (279). The changed extinction, due to axial orientation of the erythrocytes at higher flow speeds,

which may affect a continuous recording of the oxygen saturation of the blood, can be compensated for by a rather simple procedure (446).

A simple and obviously convenient constant infusion apparatus has been described, which can be built with little cost (250). An improved method for perfusion of isolated lungs of small animals has been used (38), and a rather cheap perfusion medium, which to some extent preserves vascular "reactivity" and the responsiveness of vasomotor nerves, is described (173). In perfusion experiments on dogs, horse blood and ox blood have been utilized with some success, although of course there are also serious drawbacks (98).

For the study of the smallest blood vessels a variety of methods have been described in recent years. The rat mesoappendix bioassay method, so extensively used during the last ten years, has now at long last been critically analyzed by several investigators. It is only a pity that it was not done ten years ago. It is now stated, also by members of the group who introduced the method, that there are a "number of uncertainties" and only under certain specific conditions can some degree of limited reproducibility be expected (20, 94, 467). The question is whether it is not time now seriously to reconsider the whole VDM-VEM concept, which is almost entirely based in this assay technique. With rather simple means it is possible to study circulatory events in the small vessels of the bulbar conjuctiva of human subjects (115). For some special cases the more delicate cheek pouch of the Chinese hamster is preferable to that of the generally used golden hamster (172). The phase contrast microscope can be used for relatively accurate estimations of wall thickness of small vessels *in vivo*, which otherwise is a difficult task (271).

### The Arterial Blood Pressure and the Pulse Wave

The arterial pressure and its changes in different body positions and light exercise has been measured in healthy young students (138), and there is also evidence that the neurogenic cardiovascular control in so called "nervous" circulatory disturbances is more labile than in healthy individuals (112). The changes in arterial blood pressure with increased intrathoracic pressure have been followed both in man and in animals (186). When the Riva Rocci method is compared with direct pressure measurements it is found that the indirect method generally gives somewhat lower values and that the true diastolic pressure best corresponds to the point where the muffling of the Korotkow sounds starts (32, 439), as also Burton made quite clear in his 1953 review. Other errors inherent in the Riva Rocci method have also been studied, such as the effect of increased arm volume, etc. (12, 13), or changes in the elasticity modulus of the vessels (121).

The characteristics of the pulse wave and its change with the length of the "Windkessel" have been analyzed on dogs (39), and the necessity of having well-equilibrated sphygmographs to measure pulse wave velocity reasonably correctly is illustrated (303). A mathematical analysis of the pulse wave events (290, 366), most intricate to the layman, is recommended to those

familiar with mathematical language. In quite general terms, the author has a feeling that during the years a disproportionate amount of work and significance has been put into the characteristics of the pulse wave as correlated to stroke volume, etc. Most impressive mathematical concepts have been worked out, probably of top quality from the physical-mathematical point of view. A complicated biological system such as the cardiovascular system has many variables, however, changing from moment to moment and they are almost impossible to measure in man. To overcome this obstacle "constants" are often used which in the biological system in no way are constants, and so one ends up with something with an impressive facade of scientific exactness in which many hazards may be concealed. No doubt the above may merely reflect the ignorance of the author, but nevertheless it feels good to have it said. It might be in line with these objections, however, that, for instance, oscillograms and the "oscillometric quotient" show such marked "spontaneous" fluctuations that their value for analyses of blood flow disturbances is considerably reduced (229, 255). Perhaps one is a little better off by using the "second oscillometric quotient" (210).

In model experiments some support is obtained for the old view that the movements of the arteries with the pulse may to some extent facilitate the blood flow in adjacent veins (283). Lastly, the concept of "intrinsic blood pressure" has been introduced and discussed (11). When illustrated on a model the reasoning seems to be all right, but what is really measured in the very complicated biological system is another question, and it is difficult to say what the obtained values really represent. If only the exchange across the walls should be of importance, it is a little surprising to note that, for instance, the obtained pressure value is higher in hypertensive patients.

### Biophysical Aspects of the Structure of the Blood Vessels

In a most stimulating article Burton (63) deals with the biophysical characteristics of the small vessels. The critical closing pressure concept and the possible morphological arrangement responsible for the apparent rigidity of the systemic vascular bed are discussed in detail. It is stated that evidently both the endothelial and smooth muscle cells, and, of course, the elastic tissue, are definitely distensible, and it is suggested that thin collagen fibres constitute a sort of adjustable rigid jacket, always kept tight around the vessel at all tone levels by anchoring to the smooth muscle cells. It is obvious that this cannot be obtained by a simple series connection as the distensibility of such a system should be equal to that of the smooth muscle cells themselves. Therefore it is proposed that the connection is arranged in such a way that say a ten-times increase of force is gained by allowing the muscle cells to shorten ten times more than the collagen fibrils. At first this sounds convincing and simple enough, but nevertheless it seems to be impossible for the following reasons. The smooth muscle cells are arranged in a circular way around the small vessels. If they shorten ten times more than

the collagen fibrils, which, to counteract radial distension, must also be mainly circular, it follows that the collagen fibrils then no longer are drawn tight, compared with the distensible wall components. Therefore they give no rigid support to the wall and it is mainly the distensible smooth muscle cells which are exposed to the pressure. Only at maximal dilatation, when the vessels are distended to the point where the collagen fibrils become stretched, a limit is reached where the vessel is practically nondistensible, or at such a pronounced smooth muscle tone that the blood pressure can no longer significantly distend the thickened walls from the decreased lumen. Between these two extreme points the vessels must in principle be distensible to a varying extent, and their apparent rigidity probably results from other factors.

The author some years ago (158) discussed an alternative explanation. If a given pressure causes some distension but also induces a reactive increase of smooth muscle tone, a relatively exact balance between these two opposed factors will create what looks like rigidity. Such a reactive change of smooth muscle tone may be partly myogenic (see, e.g., 66), partly a result of the fact that vascular tone also depends on the local tissue concentration of vasodilator metabolities. Any increase of pressure (with a consequent distension) will increase blood flow which, however, causes a reduction of the local concentration of vasodilator metabolites and smooth muscle tone is then somewhat increased. The opposite will happen when the pressure is lowered: smooth muscle tone, decreased by metabolite accumulation, will maintain or even increase the vascular lumen in spite of a certain elastic recoil. Recent observations (398) are interpreted in such a way and there are also older investigations which are taken to indicate such a mechanism. This is not only an academic question, because the balance between distensibility and reactive tone probably varies in different regions. This balance may be set at a new, higher level when, e.g., in hypertension, the vascular walls become hypertrophied and then less distensible for a given force (159). Also the hypertrophic growth may as such decrease the lumen. Other factors remaining constant, this purely morphological-physical change will increase peripheral resistance but still allow a normal range of vascular reactions. Thus a secondary factor that stabilizes and "organizes" the hypertensive disease may be created, once the "trigger factor(s)" initiates hypertrophic vascular changes.

The thermoelastic behaviour of aortic strips (274), the characteristics of elastic and muscular vessels to distension at various rates (359), and the influence of distension on the smooth muscle cells in isolated arteries (198, 465) have been studied. There are also reports of the change in aortic distensibility at increasing age (436) and of collagen and elastin content of various parts of the arterial tree (212). Lastly a comparative-morphological study indicates the frequent occurrence of a cartilagenous protruding ring where vascular branches leave the lumen of the main artery (435). What this means from a hemodynamic point of view is not quite clear.

The previously discussed decreases of smooth muscle tone obtained when pressure (and flow) is decreased probably explains why in dogs (and easily demonstrated also in cat limbs) it is impossible to correlate a definite critical closing pressure value to each level of vascular tone (282). Smooth muscle tone is generally very rapidly changed as soon as the environment is changed, as by a decreased blood flow; therefore it would only be possible to measure exactly the prevailing critical closing pressure by an instantaneous procedure, which can hardly be done at present. This will somewhat lessen the practical value of measurements of critical closing pressure the background of which is as yet not quite clear as regards involved mechanisms.

If the buffer nerves are cut there is an almost straight line correlation between systemic blood flow and blood pressure (281). This linear correlation is hardly any guarantee for a static condition with rigid vessels; on the contrary the previously discussed interaction of distensibility and reactive smooth muscle tone changes is probably taking place, superimposed on the changes in vasomotor fiber discharge induced when the blood flow to the vasomotor center is changed. This must cause similar, though weaker, changes in sympathetic discharge to those seen when the baro- and chemoreceptors are also able to exert their influence. The straight line correlation is probably a mere coincidence in the balance of these factors and concomitant changes in effective blood viscosity. Another study of a similar type (326) should be interpreted with such considerations in mind.

By means of an experimental and mathematical approach, the pulsatile flow in arteries with the phasic appearance of backflow has been dealt with in interesting papers (299, 456), and in model experiments it is made clear that the "paradoxical" increase of flow in collapsible tubes, obtained when a limited outflow resistance is applied, is attributable to the fact that the increased internal pressure keeps the tubes widely distended, thereby lowering their resistance to flow (364).

In perfusion of whole animals with intact vasomotor regulation it was found that it made little difference to the peripheral resistance, for example, whether the flow was pulsatile or steady (445). The circulation time is shorter in the erect position, probably attributable to a reflex constriction, with narrowing of the veins, which will increase the local flow velocity, other things being unchanged (202). The same should happen when the lungs are in the expiratory position, when their vessels are less distended and elongated (45).

High fat feeding may cause some erythrocyte aggregation and thus increase effective blood viscosity (424). The claim that the erythrocyte content varies in different major vessels could not be confirmed (206); this would also be expected provided the splenic vein is not compared with other veins nor marked differences in regional shifts in fluid across the capillary walls induced. The formation of sludges and their embolization of the

"bottlenecks" of the vascular tree have been extensively studied (381). Lastly, more general aspects on hemodynamics are outlined in some recent papers (304, 387).

### Capillary Circulation and Exchange

The fine paper of Pappenheimer *et al.* in 1951 has now been supplemented by a study of filtration, diffusion and molecular sieving through cellulose membranes (360). The results are in good agreement with the findings on the capillaries and render further support to the membrane pore theory. No evidence could be obtained of an influence of corticoids on capillary permeability (361). It seems as if quite a marked and prolonged reduction of oxygen supply is needed before definite capillary damage is induced (220). Another study dealing with the passage of dextran molecules of varying sizes indicates that, beside the capillary pores, there are also capillary "leaks" with no restriction even to the passage of very large molecules (196). Deuterium and tritium dioxides are found to pass capillary walls in the predicted way, i.e., similarly to ordinary water (82). The loss of T1824 from the blood stream has been followed in a study that also permits an evaluation of the protein exchange between plasma and extracellular fluid (389). Lastly, the influence of sodium ions on the colloid osmotic pressure and the formation of edema is discussed, and it is suggested that more marked variations in sodium concentration may significantly alter the exchange balance across the capillary walls (254).

### Arteriovenous Anastomoses

In the dog the majority of the cutaneous anastomoses are found in the feet, subserving the heat regulation (343). With an indirect method the distribution of the blood flow through these shunts and through the "metabolic" vessels is evaluated (388), but it is questionable whether with the technique used a clear-cut analysis of such a delicate nature is always possible. To judge from the passage of 100 to 140 micron spheres there must be a number of wide arteriovenous anastomoses in the stomach and the duodenum, but little is at present known about their functional significance (401).

### Venous Vessels

The control of the veins is obviously of great importance, maybe not so much for the peripheral resistance but rather for the rate of return of the blood to the pump via this adaptable "capacity section" of the vascular tree. The participation of the veins in vasomotor reflexes has been studied by Alexander (6, 7), and generally a concomitant constriction of "resistance" and "capacity" vessels is seen. This seems also to be the case in man, though in patients with postural hypotension there is little evidence of a reflex nervous influence on the veins (328). Their distensibility in man has also been studied (88). Much remains to be done in this field, however, before the

control of the veins is fully understood. The net effects on venous flow caused by changes in intra- and extravascular pressures have been analyzed, and there are here many factors to take into consideration (126, 199). If the caval veins are occluded the small azygos vein drainage seems to be barely sufficient to give a venous return to the heart large enough to keep the narcotized animal alive (85).

## Nervous Control

The present state of knowledge concerning the nervous control of the circulation has recently been discussed in a review (160), where the more important literature of later years is dealt with. The old battle about the organisation of the autonomic neuroeffector junction is still going on, with supporters both of the terminal reticulum theory (68, 422, 423) and the, for many reasons more plausible, neuroeffector unit theory (224).

Many interesting new data concerning quantitative aspects of the sympathoadrenal control of the blood vessels have been presented by Celander (78). The vessels seem to be completely dominated by their direct innervation, and the normal field of action of the adrenal medullary hormones is probably constituted by their control of certain metabolic events in cells lacking a direct sympathetic innervation, such as liver and skeletal muscle cells. Significant amounts of "overflowing" constrictor mediator occur only at extreme, probably supraphysiological discharge rates, when the vessels are already exposed to the overwhelming influence of their own constrictor fibers. The arterial wall "secretion" that can be obtained on intense reflex sympathetic discharge in dogs (111) is probably an example of such an overflow of the transmitter. Further, the fact that some norepinephrine is excreted in the urine, even after adrenal gland extirpation, is probably also a consequence of a minute overflow (142), which does not necessarily mean that in man blood concentrations high enough to elicit significant remote effects are ever reached. It has been confirmed that norepinephrine, probably contained within the constrictor fibers, is the chief catechol constituent of the tissues (310). The synthesis of the mediator seems to go on fairly rapidly as the local stores are not significantly depleted by intense sympathetic stimulation (144). If the local pH is lowered, or membrane-active drugs are given, a rather marked release of the constrictor mediator seems to take place from an isolated organ (135, 146). Whether such a direct effect on postganglionic adrenergic neurons also can occur normally is not known, but this may be less probable considering the less drastic milieu changes in the intact organism. With the rat mesoappendix assay method, already discussed, results have been obtained which are taken to indicate that the constrictor fibers exert their action by sensitizing the vessels to unknown blood-borne agents (2). Considering the limitations of the method used, this suggestion is hardly sufficient to make major changes in our present well-established views on constrictor fiber control of the effector cells. Monoamine oxidase is very widespread in the tissues and is not found only in

connection with adrenergic neuroeffector units (259); sympathetic degeneration does not significantly lower its concentration in vascular walls (62). It does not seem to be of dominant importance for the destruction at least of circulating catechols (145, 147). Surprisingly enough, no increased sensitivity of denervated vessels of the bat's wing to local application of catechols could be demonstrated (450). The cardiovascular effects of sympathectomy are discussed in a recent review (124), and it is reported that sympathectomy does not significantly influence the extent of development of collateral vessels when major arteries are ligated (447). It is fair to assume, however, that the elimination of constrictor tone will allow a bigger volume flow by way of the relaxed collateral vessels, even if their further widening and outgrowth is not affected. The changes in cardiovascular dynamics when the vasomotor system is blocked in man have been studied (31).

It is said that the discharge of the vasomotor fibers of the skeletal muscles is influenced at indirect heating (89), but it must be difficult to know with certainty whether the circulation in deeper cutaneous vessels is also completely eliminated by epinephrine iontophoresis. It seems as if the sympathetic chains carry afferent fibers, probably engaged in thermoregulation, that may reflexly influence constrictor fiber discharge in other areas (91). There is evidence that in the rabbit a considerable fraction of the cranial sympathetic fibers do not pass through the superior cervical ganglion but reach, e.g., the ear vessels by other routes from the stellate ganglion (117).

As regards the central representation of the vasomotor fibers reviews of the modern literature are available (83, 160). Marked spinal vasomotor fiber reflexes can be induced in spinal man (84). As regards higher levels the topographical relationships of the vasomotor and respiratory centers have been explored (10), and it is found that cerebellar autonomic areas may markedly influence hypothalamic sympathetic discharge (466). The cardiovascular effects of intracranial compression, which probably are caused mainly by ischemic stimulation of sympathetic centers in the brain stem, are thought to be predominantly due to circulating catechols (365). However, it is difficult to see what excludes participation of the vasomotor and cardiac sympathetic fibers as the quantitatively most important links. Convulsive shocks, through which higher sympathetic centers are also activated, are found to cause marked constriction of the smallest blood vessels in the human skin (104). In certain types of psychic activity in man, higher levels of the vasomotor fiber control are obviously activated to judge from the interference with depressor reflexes elicited from the baroceptor regions (415). In studies on patients with prefrontal leucotomy some evidence of a frontal lobe influence on vasomotor control has been gained (136). Further, the effect of anesthetics on the vasomotor responses elicitable from the cerebral cortex has been studied (272).

Our present knowledge concerning the cholinergic sympathetic vasodilator fibers has been summed up by Uvnäs (432), and these fibers are now well known. Their mesencephalic representation has been analyzed (287),

and it is confirmed that they constitute a dilator mechanism quite unrelated to the more generalized dilator effect caused by inhibition of constrictor fiber discharge (289). The distribution of cholinergic dilator fibers to the muscles of the dog has been further confirmed (462). Nevertheless, the very concept of specific sympathetic dilator fibers has been questioned in a recent study (68), but taking all available experimental work into consideration there can at present be no doubt about their existence (160).

In the salivary gland the sympathetic vasomotor fiber effect is a pure constriction, and the secondary dilatation is caused by metabolites released from the activated salivary gland (139). In addition, the vasodilator response in this gland obtained on parasympathetic stimulation is thought to be caused by a similar mechanism (232); however, it cannot as yet be definitely exluded that there really are specific parasympathetic dilator fibers besides, the more so since a distribution of such fibers to the adjacent tongue can hardly be doubted.

The functional significance of the dorsal root dilator fibers has been further studied; they are identical with the unmyelinated pain fibers and have, therefore, no true efferent function, but create a local dilatation as a response to harmful stimuli by way of axon reflex arrangements of their peripheral extensions (79). Surprisingly low discharge rates cause maximal vascular effects (80). It appears that only the smallest blood vessels are influenced (231) and the mediator is thought to be related to ATP (237). Some characteristics of axon reflexes mediating vascular responses have been studied also in man (36).

As regards the humoral link of the sympathoadrenal system, it seems, as mentioned, as if the quantitative importance of the catechol hormones for the "motor" control of the blood vessels has in general been grossly overestimated (78). Studies of the vascular effects of these hormones are also found in other publications (113, 114, 127, 128, 180, 184, 189, 413). After bleeding, norepinephrine may sometimes increase peripheral blood flow (300), probably attributable to effects on the myocardium and on blood depots. This in turn increases the diminished cardiac output and thus is followed by a reflex inhibition of a previously pronounced constrictor tone that under these circumstances overcomes the direct constrictor effect of norepinephrine. When given intrathecally, even large amounts of epinephrine have no significant effect on circulation but only on glucose metabolism, as the hormone seems to be very slowly absorbed into the blood stream (284). There is evidence that the catechol hormones to some extent are carried by the blood platelets (440). Further support for separate epinephrine and norepinephrine cells in the adrenal medulla has been presented (67, 140).

### Receptors of Importance for Vasomotor Control

The literature dealing with the characteristics of the baro- and chemoreceptors has been reviewed (228), and morphological (49) and physiological aspects (97, 101, 256, 278, 285, 295, 327) are dealt with in recent investiga-

tions. It is claimed that the baroreceptors should be unable reflexly to moderate the vascular effects of catechols as judged from the blood pressure responses (59, 230), but a more detailed analysis (e.g. in cross circulation experiments) is desirable. The result of another study, where quantitative aspects on the baroceptor control of the circulation are discussed (251), rather indicates that these receptors in the usual way counteract the pressor responses caused by injected catechols. In the toad it is found that stimulation of mechanoreceptors in the right atrium elicits a reflex vasodilatation (183), and some other studies deal also with the participation of cardiopulmonary receptors in vascular control (267, 331). In the thoracic aorta, furthermore, there is evidence of another type of mechanoreceptors, causing vasodilatation presumably by inhibition of constrictor tone (197).

### Humoral Factors

Page has discussed in a review serotonin and its cardiovascular actions (329), and further evidence that this substance can stimulate cardiopulmonary receptors has been presented (265). In the kidney serotonin seems to exert no specific effects on circulation besides its action on the vasomotor nerves (92). Pitressin has what seems to be a "specific" delayed vasodilator effect (169, 170), also demonstrable in hypertensive animals (167). The cardiovascular effects of the adenosine compounds have been studied in unanesthetized dogs (434) and the effects of histamine in cats (137). ATP is also a potent vasodilator of the limb vessels in man (122).

An $\alpha$-globulin in the plasma has been found to be the precursor of an enzyme-like factor, probably released in inflammatory conditions, which has drastic effects on capillary permeability (307, 452). The effects of trypsin on the circulation are thought to be caused by release of kallikrein (444), a factor still of unknown physiological significance. It could not be confirmed that trypsin, at least when given in tolerable amounts, has any lytic effect on thromboses (426).

Enzyme-blocking agents have been used in an attempt to analyse the reactions responsible for vascular smooth muscle activity (227). Organic redox substances, which form coloured compounds with cell enzymes, have also been tried for localisation of chemical reactions of importance for vascular reactivity (165). Lastly, the effects on human vessels of local applications of hormones have been studied (344).

Generally speaking, if physiological effects are to be evaluated it should be remembered that practically any factor, if only given in doses large enough, will affect the blood vessels, effects which maybe are never seen at normal concentrations of the substances.

### Regional Systemic Blood Flow

Many interesting aspects of regional blood flow in man, including the methods used in such studies, are discussed in a recent Ciba Foundation Symposium (469).

*Cerebral circulation.*—The distribution of the anterior chorioidal artery (69) and the arrangement of the blood supply of the hypophysis (459, 460) has been studied in man. It has also been shown that the smaller pial arteries often form "ring anastomoses" (385, 386).

To estimate cerebral blood flow radioactive krypton has been utilized instead of nitrous oxide (273), labeled erythrocytes have also been used (322, 323). Some correlation is found between the cerebral arteriogram and the volume blood flow (55, 60).

When hypotension is induced, cerebral vascular resistance is generally (153, 316, 345, 418), though not always (321), lowered. Presumably this is attributable to an increase of dilator agents in the tissue when pressure, and thus flow, is reduced, provided the vessels are not already maximally dilated. Such a mechanism is probably also responsible for the relatively unchanged cerebral blood flow when the venous pressure is raised (315). Naturally enough, the effects on cerebral function are related to the actual flow reduction rather than to the decreased blood pressure (416). With increasing age the metabolism and the blood flow are both decreased (148). In cerebral concussion the blood supply of the brain is generally increased, so the loss of consciousness is hardly induced by ischemia (58).

In animals sympathetic stimulation has only slight effects on cerebral blood flow with no evidence of a significant tonic influence of the constrictor fibers (293). It is suggested that in man sympathetic block may sometimes improve cerebral blood flow (286), but it is difficult to be sure about this because of the hazards inherent in all indirect blood flow measurements. Some morphological details in the innervation of cerebral vessels have also been dealt with (201), and the effects of pressor drugs on cerebral blood flow have been studied (317).

The characteristics of the blood-brain barrier with its creation of a specific type of restricted permeability are discussed (56) and evidence is presented indicating that the barrier to some extent is dependent on a mucopolysaccarid ground substance (225).

Lastly, a general survey of modern studies concerning cerebral hemodynamics, seen from the clinical point of view, has been presented (400).

*Coronary circulation.*—The occurrence of interarterial anastomoses (129), the distribution of the anterior descending branch of the left coronary artery (93), and the effects on the performance of different parts of the heart of obstruction of various coronary branches (301, 302) have been studied in animals.

The factors of importance for mean coronary blood flow have been analyzed (252), and among other things it is confirmed that coronary vascular tone is correlated to venous oxygen tension, which reflects tissue oxygen tension. When blood oxygen capacity is decreased (71) or arterial oxygen tension is lowered (3, 4) the coronaries are proportionally dilated until the natural limit of maximal dilatation is reached. They are also dilated by increased $CO_2$ or lowered pH (431), though not as sensitively as when oxygen

is decreased (3, 4). In other words, "a vascular reserve" created by a given inherent, basal tone is utilized under these circumstances. It is claimed that the net effect of ventricular contraction is to enhance coronary flow and that the increased flow caused by epinephrine administration is attributable to such a mechanical effect rather than to a true widening of the vessels (451). Certainly this suggestion will arouse discussion and further study.

Occlusion of the aorta does not drastically interfere with the coronary blood flow, probably because increased inflow pressure compensates for increased intramural pressure and, further, changes of vascular tone may be induced (238). If the coronary sinus is ligated coronary blood flow is significantly decreased (21), which sounds reasonable; myocardial damage may even be induced (428). As always when the nutrition is threatened anastomoses seem to be developed since arterial ligation later is less deleterious than when performed on intact animals. This only means that a reduction of the blood supply is often better tolerated when induced in stages with time for compensatory reactions in between. However, it might be dangerous to express this by saying that coronary sinus ligation "improves the nutrition of the heart." If adhesions are induced, collaterals are soon established between the coronary and the bronchopulmonary circulation (44); chronic anemia seems to stimulate the growth of coronary collaterals (130).

Lastly, an attempt has been made to isolate and study the enzyme systems of the coronary smooth muscle cells to analyse their reactions (70).

*Kidney circulation.*—In mice the glomerular blood flow can sometimes be visualized, and there are signs of some intermittence of flow (213), probably an example of inherent rhythmical smooth muscle activity, closing the arterioles when the wall tension exceeds net intravascular pressure. It has also been possible to measure directly postglomerular capillary pressure (455). The possible types of renal shunt mechanisms have been discussed (417), and quite recent observations may require a dramatic change in our views concerning intrarenal hemodynamics (334). The effects of increased hematocrit values on renal blood flow have been studied (412), while another group (353) has shown that increased protein intake is followed by increased glomerular filtration and effective plasma flow.

In "resting" conditions in dogs (209), or even after slight bleeding (181), there is no sign of a significant tonic sympathetic control of renal vessels. It is obvious, however, that in many circumstances drastic constrictor fiber effects can be elicited (24, 42, 47, 78, 133). The oliguria after severe bleeding seems to a great extent to be attributable, however, to changed tubular reabsorption (23). After bleeding, norepinephrine injections can sometimes result in an increased renal blood flow (314), probably the net result of a reflex inhibition of renal constrictor tone when arterial pressure is raised, the increased perfusion pressure as such, and the direct norepinephrine constrictor action. If there is no prevailing tone to inhibit, the direct constrictor effect may dominate. Renal neurogenic vasoconstriction seems to be more easily induced during work in patients with valvular heart disease

than in normal individuals (442, 443). There is also some evidence that general anesthesia under some circumstances may lessen the risk of drastic reflex renal vasoconstriction, possibly by its depressing effects on higher autonomic reflex centers (22).

It is suggested that alpha-hypophamine (pitocin) has a specific, though in its point of attack unknown, effect also on real blood flow (108); it will be interesting to follow further analyses. Lastly, a review of effects of circulatory changes on kidney function has been presented by Selkurt (395).

*Splanchnic and liver circulation.*—Marked reflex intestinal vasoconstriction is elicited by a variety of stimuli; epinephrine induces constriction, while ATP and acetylcholine have a dilator action (41). The effects of occlusion of the thoracic aorta on splanchnic blood flow has also been studied (8). The main outlines of splenic circulation have been discussed (477) and the depot function of the spleen further analysed in animals (40, 187, 205), and also in man (179).

The liver circulation has aroused much interest during the last year and is dealt with in a monograph (471). Morphological details concerning the liver vessels have been studied (134, 258), and methods for arterialization of the liver (154) and for gradual occlusion of the portal vein (346) have been described; the effects of blood flow restrictions on liver function have also been evaluated (347). The "hepatic vein wedge pressure" seems to correspond well with portal vein pressure (441); this method gives no evidence of portal hypertension in organic heart disease beyond that attributable to the unavoidable "back pressure" (458). The pressure in splanchnic branches of the portal vein has been directly measured in man in abdominal surgery (340), and the hepatic vein pressure has also been estimated (397). The bromsulphalein method has been further utilized (73, 74) but is unreliable when liver function is interfered with (394). Colloid radiogold can also be used instead of bromsulphalein (433). Visualization of the portal liver vessels by percutaneous injection of radiocontrast material into the spleen has been much used for the study of the arrangement of the vessels (19, 119, 393) and the streamline portal blood flow (120). The method has also been employed for estimation of portal vein and liver circulation time (132).

*Circulation in adrenal glands.*—This tissue has a considerable blood supply (214), and it is said that moderate doses of epinephrine dilate these vessels, which on the whole seem to be very resistant to catechols (294).

*Uterine and fetal circulation.*—The not too reliable $Na^{24}Cl$ clearance method has been used for approximate uterine blood flow evaluations in normal and eclamptic women (311). Evidence has been presented to show that the counter-current flow of the maternal and fetal placental circulation puts oxygen diffusion in a more favourable position (269). With an interesting method the distribution of the fetal peripheral blood flow has been studied in lambs (105).

*Circulation in skeletal muscles and skin.*—It seems now to be clear that the inflow rate of blood, as measured by plethysmography, is not significantly restricted if the venous obstruction periods are kept relatively limited (193).

The same research group (192) suggests that a moderate increase of transmural pressure induces a reactive increase of vascular tone, possibly myogenic, and no evidence of any "venivasomotor reflex" was found (337) as had been suggested previously by Gaskell and Burton (see also 160). At more intense increases of transmural pressure a marked vasodilatation of unknown nature can be obtained (191), possibly a "stress relaxation" of arterioles under excessive pressure.

The circulatory events in reactive hyperemia have been studied in man with an indirect method (296) and it is confirmed that the "blood debt repayment" is not much influenced by the magnitude of the initial blood flow level (336, 338). Probably the dilatation is mainly due to a common metabolite factor which does not seem to be identical with histamine (123). The oxygen consumption of the muscles has been measured concomitantly with the blood flow under various conditions (313), including reactive hyperemia (312). In muscular work the venous oxygen content often is seen to increase (291), which is taken to indicate that the tissue oxygen tension is not the ultimate determinant of vascular tone.

It has been said that muscle blood flow of the forearm can be relatively well-separated from the cutaneous circulation by epinephrine iontophoresis (90). The increased blood flow caused by ultrasonic energy seems to be only a matter of the increased local tissue temperature (240, 339, 362). Lastly, the hand and forearm blood flow has been studied and compared in full-term and premature children (363).

To judge from a recent study it seems as if not only the vessels of the muscles but also the cutaneous vessels are definitely dilated during fainting (411). The changes in skin blood flow induced by local temperature changes (9, 246) and intravenous saline injections of different temperatures (410) have been measured, and it appears that venous tone, under some circumstances at least, can increase on exposure to heat (448). There is a relatively good correlation between skin blood flow and local cutaneous temperature in the steady state, at least within certain limits (77, 150). The circulatory readjustments in cold environment especially in the limbs have been studied in man (57), as well as in the rabbit (280) and in some arctic animals (243); in a fine monograph by Burton & Edholm (470), our present knowledge about such problems is summed up. The effects of ultraviolet light on the cutaneous vessels have also been dealt with (177, 236, 335).

Lastly, as smoking in these days is such a widespread habit that nicotine can be looked upon almost as a "normal" humoral factor, it may be justifiably mentioned here that smoking a cigarette induces a presumably neurogenic vasoconstriction in the skin and a vasodilatation in the muscles, presumed to be caused by epinephrine released from the adrenals (373, 403).

## Pulmonary Circulation

In two recent books (472, 473) a summing up of our present knowledge concerning pulmonary circulation can be found.

*Blood pressure in the pulmonary circulation.*—Quite a number of studies

deal with the blood pressure conditions within the pulmonary vessels. The "arterial wedge pressure" corresponds relatively well with mean left atrial pressure (86, 110, 175, 318, 454) and the "pulmonary vein wedge pressure" with mean pulmonary arterial pressure (175, 454), although not always (87). Theoretically a wedge pressure must depend very much on the local vascular architecture. The more anastomoses there are to adjacent vessels just beyond the point of wedging, the nearer will the wedge pressure reflect the pressure in this very compartment of the vascular tree. With few or no interconnections it will correspond more to the pressure in the bigger vessels beyond the wedging point.

*Distensibility of pulmonary vessels.*—To judge from the pressure-volume relationships of the lung vessels, their distensibility is not too marked (270), though evidently large enough considerably to lower resistance to flow at increased transmural pressure (453).

*Pulmonary capillaries and adjacent small vessels.*—Some aspects on diffusion exchange across pulmonary capillary walls have been studied (81), and the problem of pulmonary edema as related to hydrostatic and chemical factors is also dealt with in a series of studies (27, 48, 75, 76, 245). There is some evidence that, at a given transcapillary pressure, the increased stress on the wall and its intercellular cement may induce a sudden widening of the "pores" resulting in a massive leakage including large molecules.

Further, in this part of the circulation a type of "vasomotion" with intermittence of flow can be directly observed (242). Most authors agree that the constrictor effect of oxygen shortage is due to a direct action on the vascular smooth muscles of the lungs (125, 219, 402, 421), but it has been claimed that this is probably not the case in man (155).

*Nervous control of pulmonary vessels.*—Independently of blood pH or a concomitant influence of epinephrine, sympathetic stimulation always constricts pulmonary vessels; their dilatation during work can therefore hardly be ascribed to any reversal of sympathetic action when blood composition is changed (100). A fraction of the constrictor fibers are found to cross over to the contralateral lung (99).

*Pulmonary circulation and the influence of breathing, work, bleeding, etc.*—The resistance to pulmonary blood flow is increased in inspiration (51), which as far as can be judged must mean that the hemodynamic effect of elongation of the vessels overcomes the increase of mean radius when the lung is distended. Nevertheless the blood flow increases, as the better filled right ventricle puts out an increased stroke volume. The effects on pulmonary circulation of the Valsalva test, evidently more correctly called the Bürger test, have been studied in detail (46).

In mild exercise the resistance to flow is somewhat decreased (65, 405), probably attributable both to a passive distension when increased cardiac output raises the pressure and to opening up of new channels and widening of others by vascular smooth muscle relaxation. The intermittence of flow, earlier mentioned, must mean that in any given moment a fraction of the

smallest vessels are closed. In Burton's terminology, on increase of intravascular pressure or decrease of intramural tension, or both, the vessels will no longer keep the vessels critically closed, and their corresponding capillaries are then again perfused. Something like this must happen when the pulmonary capillary surface is increased during muscular work. Further evidence has been presented to show that the lung vessels constitute a depot from which blood is easily mobilized (179). In dogs occlusion of one main pulmonary artery branch does not cause any major disturbances in circulation (16), though as for respiration things must be happening as the unperfused lung will act as a "parallel dead space." Nor has extensive embolism of the pulmonary vessels any more remarkable circulatory effects (1). The influence on the pulmonary blood flow induced by aortic occlusion has also been studied (238). The interesting and dramatic changes in pulmonary circulation following physiological closure of the ductus arteriosus after birth has been analysed both in lambs (106, 107) and guinea pigs (357, 358). Lastly, the arteriovenous dye equilibration method has been utilized to measure circulating blood volume in the lungs (354).

*Pathophysiology of pulmonary circulation.*—Pulmonary hypertension is discussed in two recent review articles (152, 476) and secondary types are contrasted to the so-called primary pulmonary hypertension (118). Analyses of different types of secondary pulmonary hypertension have also been presented (161, 203, 463, 464), and there is evidence that when the pulmonary vessels are exposed for some length of time to increased pressure hypertrophic changes, first of the media, then of the intima, occur. The flow resistance increases concomitantly (compare discussion under "Biophysical aspects," p. 162). Such changes are also seen when a high intravascular pressure is induced by left heart failure (408). In rabbits experimental pulmonary hypertension ultimately leads to arteriosclerotic changes in the lungs (26).

## Aspects of General Cardiovascular Adjustments

The concept of "cardiovascular reactivity" has been outlined in its principles (330), and as the term includes so much there may be a risk that, for instance, everything going on behind the curtain of arterial blood pressure responses is discussed in terms of "reactivity changes" without further analysis whether it is a question of changed sensitivity of the effector cells (heart or vessels), the cardiovascular receptors, or the autonomic reflex centers, etc. It should be stressed that a decreased "reactivity" of, for example, the buffer nerve reflexes, would increase a given blood pressor response to a smooth muscle stimulating drug, while a decreased "reactivity" of the effector cells themselves, would decrease this pressor response.

*Muscular work.*—The change in vascular "reactivity" during the marked adjustments of circulation taking place in muscular work has been studied (222) and also the effects of work on systemic and pulmonary blood pressure (116) and on blood flow in regions not directly engaged in the work (374).

*Respiratory influences.*—An analysis has been performed of the cardio-

vascular effects of different types of artificial respiration (14, 239), and also the effects of the Valsalva maneuver have been investigated, especially as regards nervous adjustments (276, 399).

*Hypoxia.*—Graded levels of hypoxia have of course marked cardiovascular effects, where the balance between the local dilator influence, the excitation of the vasomotor center, and the effects on the heart will change depending on the level of the hypoxia (182). The pressure in smaller veins is often raised in hypoxia with little change of central venous pressure (to begin with at least), probably due to the changed profile of the curve of pressure drop along the vascular tree when volume flow is increased through dilated resistance vessels (320). A somewhat similar study has been performed on animals made anemic by chronic bleedings (216, 406) where the most interesting question is what mechanisms are responsible for the adjustment of cardiac output to the degree of the oxygen lack in the blood.

*Narcosis.*—The effects of narcosis are of course of great interest, especially as many investigations of cardiovascular function are carried out on anesthetized animals. The changes taking place have been studied on trained animals (54, 264, 297) and chloralose seems to be preferable if one wants to maintain neurogenic control at its best performance.

*Miscellaneous.*—The circulatory changes induced by pregnancy have been discussed (64), which is also the case with some aspects of peripheral circulation in childhood (257). The changes in peripheral blood flow caused by short periods of reflex heart standstill in man have been studied (162). Curarisation of the skeletal muscles in electric shock therapy diminishes the stress laid upon the cardiovascular system by factors like massive muscular movements, Valsalva-like reactions, etc. (234), even if the pressor responses, which probably result from excitation of central sympathetic levels, remain. Lastly, to see what damage strong electrical currents can do to cardiovascular function some circulatory events have been studied in dogs during different types of electric shock application (185, 324).

## Hypothermia

When body temperature is lowered there are, of course, also changes in cardiovascular function. The arterial pressure fall is more marked if anesthesia is given (211). The coronary blood flow is relatively little influenced, as there seems to be some dilatation (33); but the myocardium seems to be more susceptible to a local obstruction of coronary vessels (396). At least to begin with, cerebral blood flow is decreased in proportion to the cerebral oxygen consumption (72, 371). Renal blood flow is decreased also (308) Moderate amounts of blood seem to be trapped away (404). There is a tendency to erythrocyte aggregation in the blood, which may obstruct smaller vessels and must, among other things, greatly diminish the available erythrocyte and capillary surfaces for oxygen and carbon dioxide exchange (174).

### Hemorrhage and Shock

Another stimulating Josiah Macy, Jr., Foundation conference on shock has been held (475) where much of the interest is focused on basic circulatory problems rather than on the shock problem as such. The significance of a changed water-salt-protein balance in shock is discussed in detail in a recent review article (370).

It is obvious that in severe shock the myocardium, too, must be suffering since, otherwise, transfusions, if given in sufficient amounts to keep up a suitable diastolic filling pressure, should always result in a good cardiac output. Often, however, death occurs in spite of repeated transfusions and with a rising venous pressure, which points to an ultimate failure of the pump. The reasons that it fails has interested a group of investigators. There is some evidence that the changed hemodynamic conditions may put the myocardium in a state of relative ischemia (131), and possibly there is also a profound change in cardiac carbohydrate metabolism *per se*, not merely the consequence of a decreased coronary flow (200). Aramine, even in amounts which do not raise peripheral resistance, seems to put the heart in a better position (379), and a net improvement of myocardial nutrition is probably created (380) where several factors cooperate.

A shock condition, started by a damage of the heart muscle, can also be created (178, 306). Evidently the decreased output is followed by a strong reflex vasoconstriction, which after some time might trap blood, especially in the splanchnic region. The lowered venous pressure head has now to drive the venous blood through the increased resistance in the liver. This tends to give a stagnation in splanchnic veins with a decreased return to the heart and thus a vicious circle may be started.

The events of torniquet shock have been further studied (260, 309, 419) and a similar condition is induced when the inferior vena cava is ligated (171).

The cerebral blood flow has been measured in man in shock (149), where compensatory mechanisms to a great extent can provide for a sufficient oxygen supply. The liver blood flow is often markedly decreased, compensated for by a better oxygen utilization, which means, however, that the liver cells have to work in a milieu of abnormally low oxygen tension (208, 247, 407).

It is thought that a neurohypophyseal factor, independent of fluid-salt balance effects, might be of importance for the resistance to bleeding (166). The protecting action of autonomic blocking agents towards drum shock can hardly be entirely explained by their specific vasomotor fiber blocking effects (223). High concentrations of VDM (rat mesappendix test) have been found in the blood of extensively injured soldiers, but the VDM content was not correlated either with the arterial pressure level or with the severity of the shock (392), which probably to many investigators did not come as a great surprise. The lessened pressor responses obtained by constrictor agents

in bled animals are probably not at all due to the occurrence of vaso depressor agents, at least to begin with, as the original sensitivity is promptly restituted by retransfusion (266). Lastly, the role of bacterial invasion in irreversible shock has been further studied (244, 390).

*Transfusion.*—The question of the relative value of intraarterial or intravenous infusion is still debated, and no apparent difference in efficiency could be observed in a series of studies (18, 409, 429). It is obvious, however, that if the heart is at, or near, a standstill when the actual transfusion is to be given, an intraarterial infusion, if given rapidly enough to build up a good perfusion pressure for the ischemic myocardium, can make all the difference in the world. It might then provide for the little extra coronary blood flow which makes the ischemic heart start again, while blood given in a vein to fill up the venous depots will here be of little or no value. On the other hand, when the heart is still in a fair condition it can hardly make any difference whether the blood is given proximally or distally to the pump in the closed system.

## Hypertension

A Ciba Foundation Symposium presents many interesting aspects on the problem of hypertension (468), and the type of hypertension seen in acute nephritis is dealt with in a recent monograph (474). A considerable number of hypertension studies from 1949 to 1952 are discussed in a review dealing with cardiovascular diseases (382, 383).

*Hypertension in man.*—Renal vascular resistance in man seems to be raised (305) already in what is thought to be the early "labile" stages of hypertension (384). As the disease may start as a purely functional, intermittent disorder, it is difficult to detect in the beginning; thus these cases may not be so early after all. Cerebral blood flow is normal; in other words, regional resistance is proportionally raised (35), but it is less likely that this is caused by constrictor fibers (95). It is said that the small cutaneous vessels in hypertension may be hyperreactive to norepinephrine (194), an interesting observation which might need further confirmation with other techniques. The errors inherent in the standard type of arterial pressure measurement can hardly significantly influence the evaluation of the occurrence of essential hypertension (342). The genetic element in hypertensive disease of man has been studied (207), and in this connection it is of interest to learn that a strain of rabbits with tendency towards spontaneous hypertension has been bred (5). Obesity seems to have a certain etiological importance (156); the effects of the diet (215) and the salt intake (188) have also been analysed.

*Experimental renal hypertension.*—This type of hypertension is still being extensively studied in many separate laboratories on various animal species. The different procedures used in rats have been evaluated (352); hamsters can also be utilized (420). The hypertension seen in coarctation of the aorta seems to be predominantly of renal origin (391). Attempts have been made to analyse the influence of neurogenic renal vasoconstriction (268),

preliminary renal ischemia (218), or the pulse pressure (217) on the establishment of renal hypertension, and in addition the influence of the diet has been studied (253). When rabbits for some length of time have had renal hypertension, elimination of the kidney factor no longer eliminates hypertension, which is thought to point to an extrarenal "maintenance factor," which takes some time to come into action (102, 103). (Is it simply due to a hypertrophic vascular change? See "Biophysical aspects . . . , " p. 162)

In some recent studies it is discussed whether a renal mechanism might normally protect against the induction of hypertension (157, 261, 262, 263) which is in some respects the opposite of the renin mechanism. Obviously much remains to be done before the role of the kidney is definitely clarified. Whether the kidney is engaged in the initiation of essential hypertension in man is completely unknown. That the kidney is probably more or less engaged secondarily in the disease when renal circulation is interfered with is another story.

Attempts have been made to determine the site of renin production (461). When renin is given subcutaneously its pressor effect is small, probably because its blood concentration then never reaches high levels (298). It seems as if a pressor factor, possibly angiotonin, can be isolated in acute nephritis (where there really are reasons to believe that renin should be released) (15). It has been suggested that angiotonin increases glomerular capillary permeability (332) though it must be difficult to exclude other factors definitely. Lastly, it appears that angiotonin and renin can be assayed on the isolated guinea pig ileum with good results (341, 349).

*Hormones, salt balance, and experimental hypertension.*—This field is still, at least to an outsider, much confused, and one has a feeling that experimental animals sometimes are loaded with hormones in concentrations quite unrelated to what can be considered to occur even in cases of definite hormonal imbalance. This, however, probably is a necessary procedure to find out the main lines in this new field. Whether essential hypertension in man has any element of hormonal dysfunction whatsoever is another question. It is still the steroids which attract the greatest interest, which is natural considering the drastic effects obtained with the hormones of the adrenal cortex. The role of hormones has been analyzed from many points of view, but space and the author's limited knowledge prevent a detailed discussion of them here, interesting as they are (163, 164, 275, 348, 368, 369, 375 to 378, 414). The sodium tolerance in different types of experimental hypertension in rats has also been studied (168). It is concluded that there is in fact little basis for the view that changes in salt and water reflect the essential disturbance in hypertensive disease or, still less, cause it (195). This of course does not prevent such changes, e.g. in vascular walls (430), from being of great interest.

*Experimental neurogenic hypertension.*—The neurogenic factor alone can hardly be responsible for well-established essential hypertension, but it may be of great importance early in the disease where nobody as yet can

really tell where the borderline between health and disease runs. Further, its influence may merely consist in intermittent periods of increased sympathetic discharge induced from the highest autonomic centers, just as some people under stress have intermittent periods of vagal hyperactivity. Such a labile factor could only be demonstrated with any certainty if it were possible to record arterial pressure changes continuously during the day in normal life. This "mean" pressure value, which constitutes the true burden for heart and vessels, might often be something quite different from what can be deduced from the intermittent pressure recordings obtained in a doctor's office. An indirect evaluation of the extent of sympathetic activity can be obtained from the measurement of urine catechols (143), but it is difficult to be sure that the same percentage of the total constrictor mediator release is always excreted in the urine during a gradual shift from health to hypertensive disease. It should also be remembered that very slight changes in sympathetic discharge activity may considerably influence peripheral resistance (160).

The hypertension seen when cerebral blood flow is interfered with is probably caused by increased sympathetic discharge (372), and the depressor effect of renal denervation, sometimes seen under such circumstances, might be caused by the lowered renal vascular resistance in combination with a possible elimination of renin production originally induced by the neurogenic constriction (367). Constriction of the carotid sinus area with rigid clamps also results in hypertension (437). Still another modification of neurogenic hypertension is obtained following buffer nerve elimination, and this type is found to be uninfluenced by ablation of cortical autonomic areas (248, 249); it is decreased by hypophysectomy (28), which probably, however, would be true with any type of hypertension. A possible intermittent neurogenic component in essential hypertension in man, which is then probably caused by a projection of the influence of daily stress on higher autonomic areas to increase their discharge, counterbalanced by a normal reflex control, has little in common with the above-mentioned experimental variants, except that the same "final common path" is utilized.

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# HEART[1,2]

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In order that this review might be kept reasonably short, a considerable number of papers had to be left out of account. Such a procedure, of course, is quite arbitrary and we should like to avoid the common practice, unhappily widespread in the world, of quoting only papers in one's native language. We therefore decided to omit all papers of predominantly clinical interest (1), for it is in precisely this field, we consider, that a lot of paper could have been saved and false conclusions avoided, if all writers read foreign periodicals more carefully.

## Electrical Manifestations of the Normal Heart

The general trend of literature seems, at least in some respect, to lay greater stress on the physical fundamentals of the electrocardiogram. Some papers are concerned with the elementary processes in single myocardial fibres, and the influence of heart rate (2, 3, 144, 145) and oxygen deficiency (4) on them. It became clear that the well-known Na- and K-permeability changes in nerve hold true for myocardial fibres as well, the overshoot being the consequence of raised Na-permeability (5). No influence, however of stretch and dilatation has been found, either on the resting and action potential of single fibres (6) or on the ECG or ventricular gradient of the total isolated heart (7).

*Theory of normal ECG.*—It seems that the vector approach to the interpretation of ECG is now widely accepted. Nevertheless an analysis which is carried down to the single fibre is, in clinical papers, a rarity. We feel that there are too many attempts at an inadequate interpretation of facts in terms of crude models, whereas an analysis of single fibre events, in the sense of Lewis' interpretation of limited potential differences, would have led to a correct and complete theory, especially in the case of endocardial and epicardial leads.

*The spread of excitation.*—There is strong evidence that the pacemaker of the heart shifts its position under various normal conditions (8, 9, 10). This holds true likewise for ectopic pacemakers of the coronary sinus (12). The starting process seems to be a local depolarization to a threshold value of about 50 mV (11, 13); vagus nerve stimulation leads to a mean local hyperpolarization of about 8 mv. (13). The course of the excitation wave could

[1] This review includes the period from June, 1954 to the end of May, 1955.

[2] The following abbreviations are used in this chapter: ECG (electrocardiography, electrocardiogram); VCG (vectorcardiography, vectorcardiogram); BCG (ballistocardiography, ballistocardiogram); KY (kymography, kymogram); EKY (electrokymography, electrokymogram); A-V (atrioventricular); ACh (acetylcholine); ATP (adenosinetriphosphate).

be followed by determining the atrioventricular conduction time in various frequencies (13a) and by inserting needle electrodes into the atrioventricular conduction system. The time relations of depolarization in the node and the Q-wave have been investigated (14). Some papers dealing with endocardial leads from the coronary sinus (15, 16), atrium (17), and ventricular cavities (18), and intramural leads (19, 20, 21) confirm the current news of the excitation process, which spreads from endocardial to epicardial surface. Over an infarcted area, the epicardial ECG equals that of the cavity (254). Independently of the location of myocardial injury the very first stages of depolarization are always involved (256). The theory of the intrinsic deflection starting at the upper inversion point should be abandoned (22). According to previous studies the myocardial excitation velocity ranges between 0.3 and meters 1.0. per sec. The trabecular zone of the right ventricle is activated first (23). The intracardiac ECG, though not without danger (24), may be helpful in localizing the catheter (25). Atrioventricular conduction has been studied with esophageal leads with which a gradual delay of the intrinsic deflection at various heights of lead position can be detected (26, 27). In a study of ventriculoatrial conduction, a 1:1 rhythm could sometimes be found in the turtle's heart. In most cases, the ventriculoatrial ratio was lower (28). Some peculiarities of abnormal atrioventricular conduction (29) and the ECG of the aortic bulb of the frog (30) have been described.

*Vectors and VCG.*—The vectocardiograms (31), presented by a mobile device (32) or stereoscopically (33, 34), and indirect calculations of mean vector direction (35 to 39), are based on the validity of the assumption that the electric source in the heart has a dipole character. This has been questioned (40, 41), a criticism which seems to be overruled by careful experiments (42, 43) and theoretical considerations (44, 45). No doubt, for the moment, the vector theory leads to a most fruitful investigation and interpretation of the ECG (46). The consistency of the membrane and dipole theory (47, 48), developed years ago (49), and the good agreement between the predictions concerning the field set up by an artificial dipole position and the real surface potential patterns (43, 50) encourage us to proceed with vector analysis, though the serious errors resulting from eccentricity (51, 51a, 52), from intrinsic components (53), and from other field distortions give rise to difficulties. The electrical "center" of the depolarization has been determined (54), but we should not forget that this center is a completely imaginary one. There are differences in the resulting vector after evaluation of various lead positions (55), which need correction in many respects (56, 57). The Wilson tetrahedron proves to be the best reference system (58). The conventional Wilson leads give rise to some difficulties concerning the "zero" potential (59, 60).

In employing the vector theory of the electrical heart field we are therefore forced to formulate rather complicated equations for the surface potential of the thorax (58, 61, 62). Investigations with thorax models (63, 64, 64a) show a large measure of correspondence between the electrical devia-

tions and the anatomy (65), and a comparison between ECG and artificial dipole fields (43) enables us to reach cautious but reasonable conclusions leading towards a theory of the ECG. The absolute value of the spatial vector may be continuously recorded by a special device (66, 67). The relative amounts of energy expended in de- and repolarization have been estimated by a procedure which is not wholly conclusive (68).

In the practical use of the VCG new types of lead positions have been used: the bipolar chest leads of Condorelli (69, 70, 71) and others (72, 73, 74), very convenient simplified three-dimensional systems with three leads (75 to 78), circular leads (79, 80), an octaxial frontal reference system (81), and leads correcting to a certain degree the field distortions of the chest (82). CF and chest leads have been investigated with regard to their relationships (83). The P-loop (84) and the main vectors of QRS and T (85, 86, 87) have been analysed and a device developed to dissect P-, QRS-, and T-loops on the screen (88). Another device gives the vector position from the data of Einthoven's triangle (89).

The Standard leads of ECG need careful correction as to the meaning of "lead vectors" (90). The VR lead should be recorded inversely to obtain a correct polarity in a continuous series of extremity records (91). The ECG has been recorded on the kymogram, which permits simultaneous electrical and mechanical observations (92). There are distortion problems of ECG-recording (93, 94, 95); questions of nomenclature (96) have been discussed and a further recommendation of standard procedures made by the American Heart Association (97). The normal standard values for spatial vectors (98), the duration of the QT which is inversely proportional to heart rate (99), and correlation between age and ECG (100, 101, 102), especially concerning ventricular gradient (100, 103) and postural hypotension (104), have been described. The limits of the normal in QRS and T areas are defined (113), showing that the angle between QRS and T even in some normal cases, may exceed 60°. The ECG may serve as a good diagnostic procedure in pregnancy since the fetal potentials can be recorded after only 20 weeks pregnancy (121). The dying fetal heart may be revealed (122).

The PQ interval seems to be depressed if P is broadened (123), of course by the well-known interference of $T_A$, which may even give distortions of QRS and T, recognizable by their behaviour in chest leads (124). A RS-T elevation probably will be normal (125). In chest leads, some of the QRS waves will be complex even in healthy persons (126). The beginning of the intrinsic deflection in unipolar extremity and chest leads may be characteristic for left heart strain (127) and negative T in $V_3$ is abnormal only in male, not in female persons (128). T has been investigated in extrasystolic beats (129) and T-U-fusion has been found (130). T and U vectors are closely related, but U seems of no special diagnostic value (132). It may therefore be a potential of dilatation. (131). As to the nature of U, no definite answer is yet available, in spite of the fact that a theory of influx dilatation as the source of U potential cannot be wholly discarded (130). A positive after-

potential has been described, provoked by epinephrine, hypopotassemia, or hypercalcemia and abolished by KCl intake (133, 134).

*ECG and mechanical events.*—Since in the last analysis mechanical factors alone will be responsible for lack of homogeneity of repolarization (135), the influence of mechanics on membrane and action potentials is of the highest clinical and theoretical interest. In single fibres, no such influence could be detected (6, 7, 136). No fixed correlation between action potential and mechanical shortening seems to exist (3, 145, 171). But the conditions in experiments with isolated fibres did not correspond to those of the ventricle, where the inner layers shorten much more and under a much higher imtramural pressure than the outer layers of myocardium. The QT-interval may vary inversely with the blood pressure amplitude (137). In the isolated atrium of rabbits the QRS interval lengthened as stretch increased and T became higher (143a). In a heart-lung-preparation, QRS was increased with augmented flow resistance (138). The areas of QRS and T are diminished during exercise, and the ventricular gradient goes down (103). During muscular work, the ECG may show very unusual patterns (139, 140, 141).

*Various influences on the ECG.*—The effect of oxygen deficiency has been studied in the human heart (142, 143) in the rabbit's atrium (143a), and in isolated single heart fibres with the glass microelectrode. There is a shortening of the action potential and loss of the plateau (4, 144). Strophanthin acts only on the damaged preparation in the familiar way (145). The ST depression may be interpreted as the consequence of the loss of plateau. The digitalis action is inhibited by atropine (146) and may, therefore, be partially of nervous origin. The effect of digitoxin is somewhat different (147). There are numerous papers dealing with ion influence on the ECG: that of lithium, which acts by changing the potassium content of the cell (148), and the direct influence of potassium (149, 150) and of acute potassium removal (152). Diminution of potassium lowers the conduction velocity while diminution of calcium increases it, and vice versa (151). A rising potassium content of the serum (153) and an increased $CO_2$ content (154) increase the T waves. The effects of lactic acid on the turtle heart (155), of x-ray irradiation on the golden hamster (156), and of epinephrine and acetylcholine action on the frog's heart [making T more negative (157)] are described. ACTH (158) and cytochrome-*c* (159) have little or no influence on the normal heart, but normalize the ECG in myocarditis (158). The T-wave flattens after high carbohydrate meals, but no consistent changes of ventricular gradient have been detected (160). In animals, we see conditioned reflexes (with drug injections as the unconditioned stimulus), indicating that some drug actions may be centrally induced (161). Hypnotic suggestion and anxiety lead to ST-depressions (162) and other events comparable to the action of epinephrine (163). Smoking does not significantly change the T-wave beyond the frequency effect, not even during general hypoxia (164, 165).

The ECG in polypneic hyperthermia (166) and in artificial hypothermia (167 to 170) is of special interest. In hypothermic dogs, the conduction veloc-

ity is reduced and, thereby, QRS increased (168). The theoretical interpretations of the author seem unsatisfactory. The influence of temperature on the action potential of single fibres reveals a constant product of rising time and conduction velocity (172) and a curious dissociation between spike and plateau (171), indicating that the depolarization during the plateau may hardly be interpreted in terms of Hodgkin's potassium theory. When the temperature is lowered, the threshold depolarization remains constant, excitation starting between 25° and 40°C., if the membrane potential equals 60 mv (173). It should be noticed that the $Q_{10}$ cannot be constant, if there is a linear correlation between temperature and conduction velocity or frequency (174).

*Fibrillation.*—Fibrillation was the subject of many detailed investigations. In spite of the laborious work done and the urgent interest in the question arising from modern surgical procedures, the results are rather meagre. Neither do we known exactly the mechanisms which elicit atrial or ventricular fibrillation nor the basic events in therapeutical effects. No doubt, fibrillation consists of a completely different sort of depolarization wave along the ventricles. This has been carefully investigated by close bipolar electrodes and high speed motion pictures in man (175). We may assume that the breakdown of the ordinary depolarization wave originates from stimuli falling into the relative refractory period, since no fibrillation was obtainable if the stimulus occurred in other phases of the cardiac cycle (176). This is confirmed at least in the great majority of cases (227 to 231). If however a stimulus is applied during a continuous infusion of acetylcholine, fibrillation occurs immediately and persists as long as this drug is infused. Atropine stops this form of fibrillation (177). Apparently there are auxiliary factors in provoking fibrillation. Epinephrine itself promotes the origin of fibrillation (178, 227, 228), increases the number of defibrillating shocks necessary to restore sinus rhythm (179), and leads to a brief enhancement of vulnerability in dogs (180). But epinephrine proves to be effective only at markedly high arterial pressures (181). Aconitine is the best and most familiar means of eliciting fibrillation and extrasystolic beats (185). It may act by blocking enzymes (182) and is counteracted by procain hydrochloride (Novocain) (217) and atropine (215). Carbon dioxide is a factor in the pathogenesis of fibrillation and cardiac arrest (183), as is $CaCl_2$ (184). It is an argument against the circus movement hypothesis that flutter is independent of ligatures in the atrial muscle (186). The potential fields of fibrillating fibres have been calculated (187).

Clinical and therapeutic problems of fibrillation and other arrhythmias have been investigated to such an extent that only a few papers with special physiological interest can be considered in this review. The electrotonic action of injury currents at the border of infarcts has been considered to be the cause of extrasystolic excitation (188). In the treatment of fibrillation, to some extent identical with that of extrasystoles and paroxysmal tachycardia, procaine and novocainamide are coming more and more to the

fore. A good survey of the recent literature may be found in reviews (189 to 192). Acetylcholine is ineffective (193, 215). Experience with new electrical defibrillators (195 to 198) and the shock therapy may be mentioned (199, 200). The strength of current necessary for defibrillation increases with the heart weight (201). Massage *per se* diminishes the chance of electrical or pharmacological defibrillation (191), producing among other things myocardial damage (202), but leads to resuscitation in cardiac arrest after procaine administration during anesthesia (203). Electric defibrillators are useful only when the electrodes are in direct contact with the fibrillating heart (even if in dogs with closed chest defibrillation were feasible) (179, 197).

In hypothermic dogs defibrillation shows some peculiarities: it is more difficult (208) epinephrine (209), and neostigmine (Prostigmine) (210, 211) were effective. Epinephrine is not the substance which induces fibrillation in hypothermic hearts, since blocking the adrenergic system has no effect (212).

The action of electric current on the heart and circulation, reviewed in two recent books (213, 214) has been studied in a series of investigations (215 to 219). The prevailing influence of the "vulnerable period" has been confirmed, in spite of an attack on it (220), which seems to miss the point. The effect of high-power currents of up to 13,000 volts has been investigated. The old suspicion that high-voltage accidents do not lead to ventricular fibrillation as easily as the currents of 110 to 220 volts seems to be based on sound evidence (216).

*Some remarks on the abnormal ECG* (221, 222).—Despite the overwhelming amount of clinical ECG work done, the reviewer wishes to express his deep scepticism of the scientific as well as the clinical value of a high percentage of these papers. There are endless reports of experience with old or new lead positions, e.g., the clinical value of the Nehb triangle (223, 224), which by the way appeared to possess no significant advantage in the diagnosis of infarcts (225, 226). If (as most observers seem to agree) the vector approach is correct and every lead combination gives records of the projection of the vector slope, we can hardly expect any advantage to result from the invention and application of new leads. The only effect will be confusion in our minds lost in this ocean of curves and waves. Sound arguments may only be found firstly for new and better reference systems, which avoid the obvious difficulties of geometrically inadequate lead positions. The Duchosal and Grishman cube seems to be better than the standard chest leads. A minimal error has been found, however, in the Wilson tetrahedron (58), and in some points of the chest, lying on a horizontal plane at the height of the center of gravity of the ventricles (227). Secondly, the possibility of recording local events by close electrode positions would be a good argument for the introduction of suitable new leads. Bipolar chest leads may yield some advantages in this respect (228). A standardization with a very few positions, however, is indispensable, if such leads are to be introduced into routine techniques. A few papers deal with basic problems of ECG and cardiology. Besides the

well-known excitatory action of aconitine (185, 189), subepicaridal injections of sodium oxalate and veratrine were found to elicit ectopic rhythms (229). This points to the predominantly unspecific nature of ectopic excitation processes, to some extent perhaps normal in so far as benign ventricular premature systoles occur in healthy persons (230). The mechanical and respiratory effects of atrial fibrillation are rather significant: myocardial hypertrophy, abnormal T, dilatation of the ventricles, hyperventilation, etc. (231).

*Blocks.*—The ECG of blocks raises several problems. Thus, we should carefully avoid interpreting the form of ECG in a morphological terminology, e.g., partial and complete bundle branch block from the duration of the QRS. "Incomplete" blocks apparently do appear in normal hearts (232, 233). Above all the inconstancy of block patterns in the same subject points very clearly to a functional source of these disturbances (234). In such inconstant block patterns, the electrical axis undergoes practically no alteration in 81 per cent (235). In the reviewer's opinion, refractory events and a slowing of conduction are some of the mechanisms involved in the production of these ECG patterns (236). The obscurities of block patterns may perhaps be the reason so many researchers every year are allured by the mysteries of Wolff-Parkinson-White syndrome. Papers on this topic are numerous. New results are lacking in most cases. In dogs it was possible to imitate the short P-R interval by injecting various substances into the atrioventricular node. "Acceleration of conduction" is said to be the reason (237). QRS, however, does not look like that of a WPW pattern. In guinea pigs, protoveratrine imitates this syndrome in a more accurate manner (238).

A detailed analysis of the ECG in infarcts could easily be obtained by applying the principles of a vector analysis of individual fibres to the various patterns of locally derived electrical events. In spite of excellent experimental work in this respect (239, 240, 242), the reviewer has the impression that none of the papers succeeded in reaching a satisfactory theory, which nevertheless may easily be derived from the results. Some theoretical difficulties will arise from the fact that, irrespective of the site of infarction, the very first vectors of Q are involved in any case (241).

The correlation of ECG with morphological or mechanical findings of the heart is of course one of the most important tests of the validity of ECG interpretation. Most of the common criteria for left hypertrophy, compared with necropsy, proved to be fairly accurate (243, 244). The more dilated the heart, the lower the potentials in the ECG (245). In right ventricular hypertrophy the correlation existing between the ECG and anatomical control was very slight, the anatomical hypertrophies in many cases having no abnormal ECG patterns (246), whereas the typical ECG pattern in the majority of cases was accompanied by hypertrophy (247, 248). The amplitude of R in $V_1$ is correlated with the state of the pulmonary circulation (249). No normal ECG was found in the presence of markedly augmented right ventricular pressures (250).

The myocardial metabolism and electrolyte content influence the ECG in a rather unspecific way. "The ECG more reliably indicates the existence rather than the type and degree of electrolyte disturbance" (251). Details of findings in hyperpotassemia (252, 253) and hypopotassemia (252 to 255) are too numerous to be reported here. A classical U-wave in hypopotassemia is remarkable (254). The prologation of the QT-interval with a shortening of the interval between Q and the second heart sound (Hegglin syndrome) was never found in experimental cardiac insufficiency in the heart-lung preparation (256). The syndrome provoked by veratrine is abolished by strophanthin (257). Many papers deal with ECG findings in relation to the surgical treatment of heart diseases. After removal of a stenosis (258, 259) or a coarctation of the aorta, R decreases and the strain pattern disappears (260), thus indicating the overwhelming influence of hemodynamic factors on the form and amplitude of the ECG.

New leads are being introduced more and more into routine clinical investigation. Bipolar leads (70, 71, 228), esophageal leads (261), and especially the VCG are being widely applied, perhaps opening up some new aspects pertaining to the dynamics of the myocardial excitation process (262). Details of the extensive work done must be omitted. A few words however should be devoted to the ventricular gradient. The theoretical meaning of this gradient remains as obscure as the nature of T. Surprisingly, however, the clinical value has met with a serious criticism (263 to 266). We should like to emphasize the fact that the gradient is the only correct means of separating the influence of QRS on T from genuine distortions in the repolarization process.

The excitability of the heart has been the subject of only a few papers. There are three types of excitability curves of the atria, if implanted electrodes are applied (267). Heart failure has no influence either on thresholds or refractory periods, conduction, or ability to respond to high driving rates in both atria and ventricles (268). The refractory period increases with decreasing temperatures (194). The heart can be driven by an artificial pacemaker for long periods (269) and even with lower than the normal sinus frequences (270). Changes of frequency are without effect on arterial pressure in such experiments (271).

## Hemodynamic and Mechanical Manifestations of the Normal Heart

*Electronmicroscopy of the heart.*—Kisch has continued his work on electronmicroscopy (272), extending his investigations of pipefish and bat (273) and comparing the results with those of flight muscles of the bumble bee (274). The sarcosomes may be artefacts in a certain sense: they cannot be found in phase contrast microscopy, but seem to be preformed as homogeneous parts of sarcoplasm (275). The influence of glucosides on morphology (276) and the nature of the intercalated discs (which seem to be cell boundaries) (277) have been discussed.

*Technical apparatus.*—An apparatus for studying the isolated heart of

cats (278), oxygenators and devices to exclude the heart temporarily from the circulation (279, 280, 281), and a hydraulically actuated diaphragm pump (282) have been described.

*The fetal heart.*—The fetal heart was the subject of several important investigations. In the last months of pregnancy the right heart grows swifter than the left (283). Newborn infants all possess approximately the same amount of heart fibres, about one-half of the adult amount (284). The process of growing has been excellently reviewed (285). The anatomical closure of the foramen ovale begins towards the end of the first week (286) in lambs. The ductus Botalli is patent up to 15 hr. after birth in humans and lambs, as is revealed by the dye dilution curves in humans (287) and murmurs in the lamb (288, 289). The patent ductus leads to a better oxygenation in the inefficient lung of the newborn by driving some of the blood a second time through the lungs (289).

*Starling's law.*—Discussion concerning the validity of Starling's law has advanced a great deal. A series of paper (290 to 299) gives an excellent picture of our present position. They nearly all agree in stating that Starling's law is by no means invalid for the normal isolated heart, but that a variety of factors also play a role in regulating the cardiac output. Among these we may list (*a*) the cardiac muscular force and manner of contration, and (*b*) neurogenic and humoral factors, mainly controlling the first factor (290). The atrial filling pressure itself is determined simultaneously with cardiac output (293). The distensibility of the relaxed chambers, the systemic blood pressure, and the heart rate (296) are, in addition, well-known components of regulation. The importance of the pulmonary blood reservoir (297, 300, 301) and the ratio of left and right ventricular outflow (299, 302 to 307) is being emphasized more and more. No doubt, Starling's law is strictly valid under rather extreme and unphysiological conditions (294), where a close relationship between stroke work (not volume!) and atrial pressure is found (290). But the more our investigation method approachs normal conditions, the more do secondary factors prevail (294). This holds true even for the frog's heart (308), which behaves very like the heart of cats in its tension-volume relationships (309).

Both ventricles develop the optimal tension with the same input volumes, and the end-systolic volume can hardly be higher than one stroke volume (310). By applying Starling's law to the left and right ventricles, one can easily explain how the two ventricles maintain the output balance under varying conditions (304). Unilateral depression of ventricular function (by ligating one of the coronary branches) leads to an augmentation of atrial pressure on the side in question (305, 503). A very important result was gained by augmenting unilaterally the outflow resistance of a completely isolated heart. Increased aortic resistance shows a feedback to the output of the right heart, which increases. Increased pulmonary resistance, however, decreases the output of the left heart, thus indicating the existence of a hitherto unknown peripheral regulating mechanism (306).

*Cardiac work.*—Cardiac output is determined with increased frequency

by means of tracers (311), the dye dilution method (312 to 317) and an analogous method using the injection of cold liquids instead of dye and observing the temperature differences in the heart (318). The technical details of the dye application (319), estimation (320) and disappearance have been discussed (321, 322), and formulas given (323, 324, 325). The normal values for humans (312, 317), for rabbits (326), and for hypothermic dogs (327) have been determined, and the standard values compared with those obtained by the Fick method, which leads to somewhat lower results (314). A modification of a bristle flowmeter, inserted in the trunk of the pulmonary artery, has been used as a new method of recording cardiac ouput (328) and improvements of measurements with the Fick principle have been described (329, 330, 331). The latter is uninfluenced by pulmonary oxidative processes (332). The influence of catheterization itself on the cardiac output is obviously doubtful: with Broemser-Ranke's method a marked increase during catheterization could be demonstrated (333), which perhaps explains some of the deviations of this method, which, on the other hand, is so much less uncomfortable for the patient than is the Fick procedure. The product of pulse pressure and heart frequency has been used to give results approximately proportional to cardiac output (334), calculation being aided by a convenient nomograph (335).

The fraction of "accumulation" in the windkessel is about 65 per cent of the total output. The rest is "flow volume" not stored in the windkessel (336). The intensity of Korotkoff's arterial sounds is no indicator of stroke volume (337). In humans, during exercise, a steady state of cardiac output is reached only within 2 to 3 min. (338, 339). Cross-circulated dogs show that neural factors are involved in regulating the cardiac output during peripherally induced muscular work (340). In experimentally-induced left to right shunts in dogs, a method for determining separately the outputs of both ventricles has been developed (341). The influence on cardiac output of cardiac tamponade (342) in dogs and of adrenalectomy in parabiotic rats has been investigated (343). Hexamethonium lowers the cardiac output in humans parallel to a diminution of peripheral resistance (344). In simultaneous records, the aortic pressure and the curve of ejection have been investigated in cadaver hearts. The cardiac output at each instant of the systole could be calculated from the contour of the pressure curve (345).

Anoxia and anemia influence the cardiac output (346, 482), and lead in animals to an increase of work and output, if the oxygen saturation is lowered beyond a level of 40 to 60 per cent (347) and the hemoglobin below 7.5 gm. per cent (348). The output is closely related to the oxygen capacity of the blood (349, 502). In controlled hypotonia, stroke volume and cardiac output remain nearly unchanged (350). Chloroform impairs acutely the work of both ventricles, in the left more than in the right (351).

The pressure events in the segments of the heart have been recorded using various methods. The classical method of venous catheterization (352) has been modified by inserting needles into the left atrium, and by passing a

catheter through the needle into the ventricle (353, 354, 355). Endoscopical punctures through the bronchi into the pulmonic artery (356) and atria (357, 358, 359), and puncture of the atria through the esophageal wall (360) or insertion of pressure recording devices into the esophagus to pick up the atrial pressure curves (361, 362, 363) seem to have been developed into routine techniques. Concerning the events in the atria, pressure curves of both sides on patients with interatrial septum defects permitted the identification of pressure gradient curves significant for these defects (364). Massive injections of fluid into the brachial vein do not lead to increases in atrial pressure (365) but cause complicated reflux phenomena (366). The influence of atrial contraction on arterial pressure in atrioventricular block (367), two types of insufficiency in isolated atria (368), and a detailed description of oscillating blood movement in the capillaries of the sinus venosus in the dying heart may be mentioned (369). The latency between Q and the rise of right ventricular pressure is equal to about 0.048 sec. (371) in records made using the Condorelli method (370, 371, 272). These also reveal the time relations between the ECG, heart sounds, and pressure curves (371) or carotid pulse (372). The isometric phase of the right ventricle lasts for about 0.032 sec. in normal hearts (373). Standard values of the various time sequences are given (372).

The pericardium plays an important role in limiting the filling rates and volumes in the presence of ventricular dilatation. A dilating left ventricle, for instance automatically restricts the filling volume of the right ventricle, and the pulmonary pressure is reduced (374).

*Respiration and cardiac activity.*—During the Valsalva procedure, the systemic as well as the pulmonary pressures, venous and arterial, are increased (374a) to the amount of the lung pressure (375). The chest behaves like a closed system in this respect (375). The inspiratory augmentation of venous return is responsible for the increase in pulmonary artery flow during inspiration (376). In artificial respiration, the positive-negative pressure method gives optimal circulatory results (377, 378). The diastolic volume of the heart depends decisively on the posture and should be determined only in a supine position (379).

*The nervous control of the heart.*—The anatomy of heart nerves in dogs has been described (380). Denervation elevates the cardiac rates to about 140 per min. in dogs, independently of body weight, breed, or sex. Epinephrine further increases the denervated heart frequency (381). The effect of vagal stimulation (382) and the normal tonic control of both vagus and sympathetic (383, 384) lead us to the conclusion that the sinus is more strongly controlled by the autonomic system than any other regions of the heart. Hypercapnia favors vagal cardiac arrest (385); acetylcholine potentiates, while epinephrine inhibits the vagal effect (386). Veratramine appears to be an antagonist of epinephrine and similar compounds (387). This may be part of the so-called Bezold-Jarisch effect. The existence of a Bainbridge reflex, seriously doubted years ago, seems to have been verified: rapid in-

travenous infusions [which do not always raise the atrial pressures! (365)] lead to an increase of heart rate if the initial rate is low, and to a decrease if it is high (388). The connection between pulmonary receptors, whose chemical excitation leads to a respiratory standstill, and the well-known Bezold-Jarisch reflex is, to say the least, uncertain (389).

*Electrokymography.*—The EKY, after its first development in Germany just two decades ago (390, 391), is still a favourite subject of research. It nevertheless seems to the reviewer that the interpretation of these curves involves many uncertainties, especially in all questions of latency time, e.g., in branch blocks (392). Details of the EKY, described in cases of alternans (393), aortic insufficiency (394), and mitral valve disease (395 to 399), mostly of artial movements, are not suitable for brief review. The EKY seems to be a fairly good index of mitral regurgitation (395).

*Ballistocardiography* (400, 401).—The application of the BCG is widening at a tremendous speed. The peculiar pleasure taken in recording curves obscure in meaning and impossible to evaluate with any degree of certainty for the benefit of our patients is difficult to understand. In this respect, the BCG even overrules the ECG. "Little help will be gained from the BCG in making cardiac diagnosis of the anatomic type", confesses Starr in an excellent review, but some insight into cardiac fatigue, reserve, failure, and myocardial weakness may be acquired through evaluation of the records (401). We are still in a state of early experimentation (402). As a matter of fact, the amplitude of the IJ-segment has a highly significant correlation to body surface area in the longitudinal (not in the transverse) BCG (403), and hexamethonium augments not only the stroke volume but likewise the IJ-amplitude (434). Imitations of cardiac systole at necropsy by simultaneous injections of blood into the aorta and pulmonary artery revealed a high degree of correlation between amplitude of BCG and cardiac output. Nevertheless, Starr's conclusion was that a factor of force mainly determines the slope of the curves (404). Quantitative measurements (405) and correlations with cardiac output *in vivo* (406) are alike impossible. This is strongly emphasized by the fact that complete ligature of both caval veins actually results in an increase in the amplitude of the BCG. Therefore, the action of the heart muscle itself must be the immediate source of the oscillations (407, 408). This conclusion is supported by the dependence of the BCG on the position of the heart, not on the output, in a spatial BCG (410) called the "vector-BCG" (409). Some technical improvements (410 to 418), e.g., records with a capacitance receptor unit (410, 411), a mercury bed (412, 413), thoracic BCG by applying the transducer platform to the thorax (414), photoelectric BCG (415), electronically eliminated body vibrations (416), and investigations concerning the influence of damping (418), may be mentioned. Simultaneous records with displacement, velocity, and acceleration BCG will be of interest (419). A quantitative evaluation by means of a correct calibration seems needless or impossible (420, 421, 422). Papers describing the normal values of the components (423), the influence of age (424, 429), body size and composition (424), and the relations to the ECG, heart sounds (425), and pulse

waves (426), and calculations of Bazett's constant $k$ (427) have been published, and the H-wave has proved to be produced by the summation of an atrial and a ventricular component (428). Several papers on the BCG of children remain to be mentioned (430, 431, 432). Hexamethonium considerably alters the I and J spike (433), especially in hypertensive patients (434).

The clinical applications of the BCG are outside the scope of this review. Numerous conclusions in these papers seem rather doubtful to the reviewer. In many cases, the BCG enables us to detect cardiovascular damage earlier than does the ECG (435 to 438) naturally enough, since only the BCG is a mechanically operating system! We may assume that patients with abnormal BCG are suffering from organic diseases (439), but even this is no more than a hypothesis.

*Rheocardiography.*—Holzer-Polzer's method for measuring the electrical resistance of the chest seems to yield very complex results (440) and difficulties in its interpretation (441). The value of this method is apparently restricted to application to peripheral vascular areas.

## The Heart Sounds

The standardization of methods for the study of the heart sounds (442, 443, 444) has been the aim of international meetings (445). Methods of calibration (446, 447) and a method of recording the envelope curve of the sound intensity (sonvelography, 448) have been developed and adapted to clinical purposes. Frequency analysis plays a more and more important role: mechanical and electronic analysers are used and the records described in detail (449 to 453) in their intensity-frequency relations (452, 453) and in the time distribution of the frequency spectrum (450). Intracardiac sounds have been recorded and indicate a certain amount of turbulence inside the heart (451). The functional character of some diastolic murmurs has been proved by necropsy (454) and by experimental approach (455). Functional systolic murmurs seem to be accompanied by high stroke volumes (456). The role of pericardium in the production of a third (457) or early diastolic sound (458) is obvious: the sudden halting of a rapid diastolic filling seems to be the cause (458). The loudness of the first sound increases with increasing P-Q-interval (459, 460) and with prolonged diastole (461) in atrioventricular blocks. Apparently, the position of the valves in the moment of rising ventricular pressure is decisive (460, 462). Besides this, the sound intensity is related to the velocity of contraction, not to the stroke volume or contraction power (463). The atrial sound is a filling sound of the ventricles (464); both sounds are often split even in normal persons, whether due to asynchronous closure of the left and right valves (465, 466) or not (467) seems uncertain. The first sound is delayed in ectopic beats (468).

## The Metabolism of the Heart (469)

The myocardium possesses an astonishing versatility in the use of its fuel supply and utilizes glucose, lactate, pyruvate, fatty acids, ketone bodies, and amino acids (470, 471). The importance of pyruvate for heart metab-

olism lies in its strategic position as the last link between carbohydrate intermediaries and acetyl-coenzymes A (470). After hemorrhagic shock, the heart's extraction of several fuel stuffs is reduced (472). In anoxia, the glycogen content, especially the trichloracetic-acid-soluble fraction, is diminished (473). Phosphorylation properties (474), ATP splitting of the myocardium (475), and the actomyosin content of normal (476) and failing hearts (477) were investigated. The ageing heart shows peculiarities in its chemical composition (478). The oxygen uptake of the conducting tissue is significantly less than that of other myocardial structures (479). Epinephrine, in humans, is said to constitute an important factor in congestive heart failure, because (as the author concludes) epinephrine concentration is highest in failing hearts (480). This may be, however, merely the consequence of liberation of epinephrine by the stressing impulses of tonic sympathetic nerve! Epinephrine is considered to waste a great deal of oxygen and to impair efficiency (480).

Some papers deal with specific metabolic effects of various heart diseases. In diabetic dogs and patients, the usage and extraction of several metabolic materials (ketones, lactate, pyruvate, etc.) is significantly changed (481), and during oligemia and the normovolemic phase of hemorrhagic shock, similar events occur (482). In all cases the assumption of specific metabolic effects nevertheless seems quite arbitrary. Most probably every heart behaves in a similar manner, if the equilibrium of its metabolic processes is shifted to an extreme position. Iodoacetate inhibits the contraction by interfering with metabolism (483). The ATP content of the heart rises during exercise after treatment with this compound (484), and ATP influences the efficiency of the heart (485). Phosphate compounds improve the process of recovery (486).

The part played by acetylcholine in the myocardial metabolism has been investigated intensively. The content of acetylcholine (ACh) in several parts of the heart has been determined (487 to 490), the liberation during activity controlled; ACh appears only in traces if 2,4-dinitrophenol is present in the perfusing fluid (491). The liberated ACh stems from the myocardial fibres and is not related to the pacemaker (492). Excitation of the vagus nerve does not change the ACh content of atria and ventricles (493). The release and combination of ACh with the receptor protein of cholinesterase may be the process which normally accelerates the ion movement across membranes (494). ACh perhaps has some influence on the plateau of the action potential (495), but other authors maintain that it has none (496).

The cholinesterase activity is higher in the right than in the left atrium (497) and is completely absent in pure myocardium (498). It is found solely in parts of the heart with a negative chronotropic effect (499). Esterase preparations induce blocks and change the mechanical activity of the heart (500).

### Various Influences on the Heart

*Influence of hormones.*—Norepinephrine (1 to 30 μg per kg.) leads to increased cardiac output in dogs, with the optimum dose at 5 to 10 μg. The

metabolism increases proportionally (505). The action of epinephrine is diphasic (506). This effect, like that of acetylcholine depends on the initial condition of the heart and may be reversed by cooling (507). In hemorrhagic dogs, norepinephrine leads to an increased cardiac output (508). Histamine has apparently no significance as a natural evocator of cardiac reaction (509). Deoxycorticosterone acetate restores heart weight after hypophysectomy (510).

*Glycosides.*—The action of glycosides on the heart has been the subject of a considerable number of papers (511 to 532). These papers deal with the cumulative effect (511), the fact that digitoxine does not elicit extrasystolic beats like strophanthin (512), the stimulation of respiration by various glycosides (513), the reactivation of inactive glycosides by insulin (514), reduction of viscosity of actomyosin (516), reduction of circulating blood volume (515), the diphasic (at first activating, in larger doses depressing) action on ganglionic transmission (517), and the relationship of the sugar moieties to the toxicity (518). Digoxin exerts its influence on the isometric and ejection phases 5 min. after application (519). Injection of strophanthin into the third cerebral ventricle caused cardiac irregularities, similar to those seen in cases of clinical digitalis toxicity (520). The lethal dose in rats depends on their age (521). Strophanthin augments the weight of the adrenals (522) and inhibits fatigue by dinitrophenol (523). Ouabain increases the tone of papillary muscle (524); its toxicity is reduced by physostigmine and vice versa (525). In adrenalectomized rats an increased sensitivity to ouabain was observed (526). Strophanthin elicits extrasystoles after aconitine treatment (527), an effect which is abolished by novocain and quinine (528). Lanatoside C exerts its cardiotonic effect in dinitrophenol (DNP) failure without inhibiting the metabolic stimulation of DNP (529). Quinidine was found to have a profound inhibitory effect on the contractility of the cat's papillary muscle (533).

*Hypothermia.*—There are several papers dealing with the surgical problem of "hibernation" or, to avoid this completely misleading expression, of hypothermia (534 to 541). The influence of hypothermia on fibrillation (206 to 212) has already been mentioned. In rats, cardiac arrest lasting up to 44 min. in hypothermia was compatible with survival for several weeks up to the time of publication. Periods of arrest up to one hour showed limited survival time (537). In dogs, rewarming brought about a drastic reduction in the activity of circulatory reflexes (538), cooling a lowering of the heart rate in accordance with van't Hoff's rule (539), and a decrease of cardiac output parallel to the heart rate (540). Hypoxia is not a factor leading to fibrillation during severe hypothermia (541).

### Some Remarks on Physiological Problems in Abnormal Hearts

The enormous literature on the heart can only be reviewed with some degree of thoroughness if we restrict ourselves to the physiological findings, omitting all papers of more clinical interest. Some reviews may be helpful guides to the recent clinical literature (352, 542 to 549).

Some anatomical results will be of interest. Oxygen deficiency causes septal defects in the embryo of the chicken (550). This bears out the fact that congenital heart diseases are frequently found in the children of women with hormonal disturbances (551), which perhaps mean nothing else than hypoxia. Heavy exercise in guinea pigs was carried out with better efficiency in those animals which had the less hypertrophic hearts. Heart weight and hypertrophy of hypophysis are significantly correlated (552). The lipoid content of hypertrophic heart has been analyzed (553). Clinical comments on dextroposition of the heart (554) are given, and an idiopathic cardiac hypertrophy has been described (555, 556).

The magnitude of the heart chambers may be determined fairly accurately by the position of the catheter in the x-ray picture (557). In a model, the unidirectional transport of blood could be demonstrated without functioning valves, as in cases of combined mitral and aortic stenotic insufficiency (558).

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# THE KIDNEY[1,2]

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Metabolic activities of the kidney formed the subject of an extensive review by Drury (1) in the previous volume of the *Annual Review of Physiology* and are not considered in the present review. Among relevant books recently published in connection with renal physiology may be included the *Fifth Macy Foundation Symposium on Renal Function* (2), the *Ciba Foundation Symposium on the Kidney* (3), *Reflections on Renal Function* by Robinson (4), a sixth edition of Gamble's *Chemical Anatomy, Physiology and Pathology of Estracellular Fluid* (5), the *Third Freiburg Symposium on Pathological Physiology and Clinical Aspects of the Secretion of the Kidney*, edited by Frey (6), the *Symposium of the Society for Experimental Biology on Active Transport and Secretion* (7), and a monograph on *Renal Clearance Investigations in Children* by Friederiszick (8). Attention should also be drawn to the extensive and important review on *Sodium Excretion in the Mammalian Kidney* by Selkurt (9), to the lecture on *Body Water Control* by Bull (10), and to the review on the *Active Transport of Water in Living Systems* by Robinson (99).

## Autoregulation of Blood Flow and Glomerular Filtration Rate

That the renal blood flow is relatively refractory to changes in pressure in the renal artery has been known for many decades and has been the subject of numerous reviews and studies including recently Selkurt (11) and Sellwood & Verney (12). The latter found "no certain changes in plasma flow" when the pressure was raised from 120 mm. Hg to 195 mm. Hg by compressing the carotid arteries in an unanesthetized dog, confirming the earlier observations of Forster & Maes (13) in unaesthetized rabbits. In anesthetized dogs Lochner & Ochwadt (14) and Miles *et al.* (15) found regulation of blood flow during changes of renal arterial pressure produced by a pump nearly as good, and even in the pump-lung-kidney preparation [Winton (16)] the change in flow is remarkably small compared with that in other organs. There is general agreement, though not without exception [Brull & Louis-Bar (17)], that this autoregulation is an intrinsic property of the kidney, not primarily dependant on external influences, e.g., nervous or hormonal concomitants of the changes in arterial pressure. Many of the same authors have shown that glomerular filtration rate also varies very little with arterial pressure, certainly much less than might be anticipated in

[1] The survey of the literature pertaining to this review was completed in June, 1955.

[2] The following abbreviations are used in this chapter: GFR (glomerular filtration rate); PAH (*p*-aminohippurate); RPF (renal plasma flow); Tm (Tubular maximum); DCA (deoxycorticosterone acetate).

any mechanism to which the physical properties of a filtration process applied in any simple way. Most authors have realized that the increase in filtration fraction with arterial pressure must be comparatively small, and that increase in blood viscosity in the *vasa efferentia* attributable to this must be too small to account for more than a small part of the large increase in resistance in the renal vascular bed with rise in pressure (16). Seeking to find a single explanation for the autoregulation of blood flow and of glomerular filtration rate, they have postulated a vasoconstrictor control in some part of the preglomerular circulation, though the problem as to what structure in the kidney, sensitive to pressure, immediately instigates the vasoconstrictor response is usually evaded.

The latter problem can be approached by studying the effects on autoregulation of agents known to prevent contraction in plain muscle, and therefore of arterioles, but the results of such studies have yielded conflicting results. Cyanide is said (14, 15) to abolish autoregulation irreversibly, and procaine to do so reversibly (14). In "dead" kidneys there is no autoregulation (14). On the other hand the autoregulation in the pump-lung-kidney preparation of the dog is hardly affected by concentrations of chloral hydrate which render plain muscle unresponsive to powerful constrictor agents (16). Nor is this autoregulation abolished by cooling the whole system to 3°C., at which temperature mammalian plain muscle is unresponsive, and the blood flow through the kidney has fallen, as a result of increase in viscosity just as much, proportionately, as it does for the same change of temperature in a glass tube, indicating absence of vasoconstrictor or dilator change (16).

A different approach to the localization of the renal element sensitive to arterial pressure change has been made by Lochner & Ochwadt (14). It has long been known (18) that whereas a fall in arteriovenous pressure difference produces little fall in renal blood flow when it is caused by decline in arterial pressure, it produces a substantial fall when it results from rise in venous pressure, such that the flow is approximately proportional to the pressure head applied to the kidney. When a dog kidney is perfused with blood by a pump with constant ouput, a given rise in venous pressure will, accordingly, produce a disproportionately larger rise in arterial pressure, the magnitude of the disproportion indicating the degree of autoregulation. This increase in renal vascular resistance fails to appear, however, if the extrarenal pressure is raised at the same time as the venous pressure (14), and it is concluded that autoregulation becomes manifest only when there is a change of pressure between the inside and outside of the blood vessels, which presumably constrict in response to the stretch.

The vasoconstrictor theory of autoregulation, whether it involves immediate response to pressure of the blood vessels concerned or reflex response to receptors elsewhere in the kidney, clearly presents inherent difficulties of quantitative analysis, and the same holds for the theory that attributes the

phenomenon to metabolic changes such as change in oxygen supply (11). The solution of the problem of autoregulation has perhaps been made needlessly difficult by the temptation, in an age of statistical jargon, to report small changes of flow or filtration rate as not significantly different from zero in observations depending on clearance measurements, which under good steady-state conditions, chemical and physical, involve uncertainties of 5 to 10 per cent. In fact all workers have found increases of both blood flow and glomerular filtration rate with increase in pressure, and it might well be that the values from which their observations would deviate least would involve, say, a 5 per cent increase in blood flow and a 10 per cent increase in filtration rate, for a 50 per cent increase in arterial pressure. Whatever the true values are (and the mean changes are more likely to be greater than less than those indicated) it is exceedingly unlikely that autoregulation involves no changes in blood flow or filtration, as is sometimes implied.

An exciting and highly original advance in our knowledge and understanding of autoregulation has been initiated by Pappenheimer & Kinter (19). They suggest that autoregulation as discussed above and a number of other puzzling and unique properties of the kidney could become explicable if a measure of separation of the plasma and cells of the blood within the renal vascular system could be postulated, the plasma-rich fraction passing through the glomerular-peritubular vessels, and the cell-rich fraction passing through shunts possibly in the juxtamedullary region of the kidney. In support of such an hypothesis they have shown that the proportion of cells to plasma passing through the superficial layer of the cortex is only about one-half that in the blood in the renal artery and vein. To this end they prepared blood in which $P^{32}$ had been incorporated in the phospholipids of both cells and plasma by biosynthesis in the livers of donor animals. The active cells and plasma were separated and each combined with normal inactive plasma or cells as required to obtain two kinds of blood with the same hematocrit and the same total activity, one with the activity in the cells, the other with it in the plasma. The radiation over the surface of the kidney was less by a ratio of 1:2 when blood with active cells perfused the kidney than when blood with active plasma was employed, depth of penetration of the radiation concerned being less than 5 mm. A second kind of experimental approach to this two-circuit hypothesis involved a study of the relation between renal extraction ratios of p-aminohippurate (PAH) and iodopyracet (Diodrast) and the hematocrit value of the blood. In anesthetized cats and dogs when few cells were present the extraction ratios fell to 30 to 50 per cent although the Tm values were fully maintained when the animals were breathing oxygen. It is suggested that in the absence of cells some of the plasma has access to the short circuits normally occupied by cells, and that the proportion of the plasma perfusing the peritubular capillaries is thus reduced with a corresponding reduction in the fraction of PAH or Diodrast which can be removed by the tubule cells.

Such a two-circuit theory could explain autoregulation of blood flow through the kidney if there were an increase in the degree of cell-plasma separation with increase in arterial pressure. This would be effective particularly because it is known that the viscosity of blood changes very rapidly with change in cell concentration when the absolute concentration is high (say 80 per cent as might be expected in the short circuits), though it changes only slowly with cell concentration at values normally found in the systemic circulation. Such an explanation is readily accessible to experimental tests, and it may perhaps be anticipated that the authors concerned will demonstrate that the autoregulation in fact fails in the absence of a suitable red cell content of the blood supplied to the renal artery.

This cell separation theory could, moreover, explain a further remarkable and possibly unique feature of the kidney which had been shown earlier, namely its relatively low dynamic hematocrit. Gibson *et al.* (20) analyzed the blood content of dog's kidneys, the erythrocytes in terms of $Fe^{59}$, and the plasma albumen in terms of $I^{131}$ or T1824. The hematocrit in the kidney was only 0.35 that in venous blood. Allen & Reeve (21) found a corresponding value in the dog's kidney of 0.49 that of the venous blood after clamping the renal pedicle in the dog and thereby including not only the blood in the smaller vessels but also that in the larger vessels. Lewis *et al.* (22) found corresponding values in rat's kidneys of 0.73 that in systemic blood. These low dynamic hematocrit values recall the old observations of Fåhraeus (23), who attributed a comparatively smaller fall in dynamic hematocrit of blood flowing through glass capillary tubes to axial concentration of the cells, the cells passing through the tube more quickly than the plasma. This observation and its explanation, even if confirmed, could hardly be applied directly to a branching vascular system with vessels as small as the arterioles and capillaries in the kidney. A somewhat similar explanation of the low dynamic hematocrit might, however, be based on the more rapid passage of the cells through the shunts than of the plasma through glomerular-peritubular circulation. For example, if arterial blood containing 40 per cent cells divides into a glomerular-peritubular vessel system containing about 10 per cent cells (say 50 ml. plasma and 5 ml. cells per min.) and a shunt system containing about 80 per cent cells (say 10 ml. plasma and 35 ml. cells per min.) and the rates of flow in the two circuits correspond to the volumes suggested, the dynamic hematocrit of the kidney as a whole would be the same as that of arterial blood. But if the glomerular flow remains unchanged while the shunt system flow becomes twice as fast, the volume of cell-rich blood in that system at any given moment must be only one-half as great. Hence the dynamic hematocrit of the kidney as a whole would fall from 40 per cent to 29 per cent. If the two-circuit theory is to explain autoregulation of blood flow in terms of an increased separation of cells and plasma at increased pressures, the dynamic hematocrit in the kidney should therefore fall at increased pressures, a consequence easily amenable to experimental test.

Yet another exceptional property of the kidney hitherto quite unexplained may become intelligible in terms of this two-circuit conception of the renal circulation. In most organs the arteriovenous oxygen difference varies roughly inversely with the blood flow to keep the oxygen consumption of the tissue fairly constant. It has long been known that in the kidney the arteriovenous oxygen difference is practically independent of blood flow within the range of pressure, 80 mm. to 200 mm. Hg covered by autoregulation (16). This autoregulation of arteriovenous oxygen difference, like that of blood flow, disappears below about 60 mm. Hg when the kidney behaves, in these respects, like other organs. Moreover, the arteriovenous oxygen difference in the kidney is exceptionally low, a circumstance which might be expected if most of the cells in the venous blood has passed through shunts without an opportunity of losing oxygen to the tissues.

Two independent photoelectric studies of the renal circulation in anesthetized dogs have appeared. In neither was a possible separation of cells and plasma recognized, but it happens that one revealed the transfer of the cells and the other that of the plasma. Kramer (24) applied his oximeter technique to the cortex and medulla and thereby found values for the speed of passage of red cells from the renal artery to the cortex and to the vein. He also estimated the blood content (as he thought), but strictly the cell content, of the cortex and medulla under the influence of various disturbances. Epinephrine reduced the renal blood flow, directly measured, to 10 per cent of the normal and produced maximal pallor of the cortex. An even greater reduction in blood flow resulting from anoxia produced only a 50 per cent reduction in cell content of the cortex. Comparable reduction in blood flow, produced by reducing renal arterial pressure mechanically, reduced the cell content of the cortex even less. In many circumstances pallor of the cortex was associated with congestion of the medulla. All the powerful agents studied produced, however, rather drastic effects on the kidney, outside what may be regarded as the physiological range.

Lochner & Ochwadt (14) have studied the times of appearance of Evans blue (T1824) after sudden injections into the renal artery, and thus followed the transfer of the plasma through the kidney. Kramer (24) found the shortest time for passage of cells from artery to vein averaged 1.9 sec. Lochner & Ochwadt found the shortest time for passage of plasma 2.1 sec. Both sets of authors used anesthetized dogs. In two series of experiments performed in different laboratories no significance can, however, be attached to the small difference between these values. Lochner & Ochwadt also studied the average time taken by the plasma to pass through the kidney and found it to be three or four times as long as the time of first appearance of dye in the vein. They calculated the blood content of the kidney as the product of the average arteriovenous circulation time and the blood flow, directly measured. Thus they estimated the blood content of the kidney at 23 per cent of the kidney weight at normal arterial pressures. At higher arterial

pressures the blood (i.e., plasma) volume in the kidney may be about 10 per cent less than at low arterial pressures, and the authors attribute this finding to vasoconstriction at high pressures, corresponding to the vasoconstriction which they believe prevents the rise in blood flow with increase in arterial pressure.

Renal autoregulation of blood flow and glomerular filtration rate in respect to changes in arterial pressure does not, it would seem, exhaust the virtuosity of the kidney for control. Other classes of autoregulation have been claimed, particularly the failure of red blood cell concentration, within limits, to produce changes in blood flow comparable with the changes in viscosity of the blood, and the failure both of this change in the blood and of changes in plasma protein concentration to produce comparable change in glomerular filtration rate.

By injecting blood or packed cells from suitable donor dogs Spencer (25, 26) induced varying degrees of acute polycythemia in recipient dogs, both anesthetized and unanesthetized. Comparatively large changes in hematocrit, for example, from 36 per cent to 62 per cent, could be produced in this way, and the corresponding change in apparent relative viscosity of the blood would, according to Whittaker & Winton (27) have been from 1.92 to 2.89 measured in a dog hind limb. In the kidney there was no important fall in blood flow, compared with the 60 per cent or so fall which would be expected in the hind limb. The renal plasma flow, derived from PAH estimations by the Fick principle, fell substantially, but this fall was compensated by the rise in the hematocrit so as to produce little resultant change in blood flow in unanesthetized dogs though there was a fall, about 14 per cent, in anesthetized animals. Similar increases in corpuscular concentration changed the creatinine clearances very little but sometimes increased them. Thus, the filtration fraction always increased with corresponding increase in the mean glomerular colloid osmotic pressure; presumably, therefore, the glomerular capillary hydrostatic pressure increased. Sympathectomy, so that the kidneys were effectively denervated, had little effect on these reactions which, like the more familar aspects of autoregulation, appear to be intrinsic to the kidney.

Spencer explains his results in terms of a preglomerular vasodilator mechanism which responds to increase in corpuscular concentration so as to maintain almost constant blood flow and glomerular filtration rate (GFR), possibly the same mechanism as that concerned in the responses to changes in arterial pressure. His tables appear to indicate a rather consistent and considerable fall in PAH extraction ratio after the injections from the donor dogs which produced polycythemia; as the author does not discuss this effect it may be of no significance, but it might also suggest the action of some toxic constituent of the injected fluid with possibly unforeseen further effects of the renal circulation.

Autoregulation of glomerular filtration rate with respect to changes in

colloid osmotic pressure of the plasma has frequently been both described and refuted in various species. In unanesthetized bitches Bojeson (28) varied the oncotic pressure upwards by injection of plasma proteins and downwards by injection of Ringer's solution over a range of 7.5 mm. Hg. He found no changes in the inulin or Diodrast clearances. A fall in oncotic pressure increased the output of water, sodium, and potassium. Sellwood & Verney (12) on the other hand demonstrated a 20 per cent increase in exogenous creatinine clearances is unanesthetized bitches after administration of 2.5 to 3.5 per cent of body weight of water or isotonic saline. O'Connor (29) likewise studied the effects of administration of saline in unanesthetized bitches on water, sodium, and chloride output, measuring the changes in total solid content of the plasma as an indication of change in plasma protein concentration. He adopts the values of Wesson *et al.* (30) for the change in GFR with plasma oncotic pressure, i.e. a rise of 1.15 cc./min. for a fall of 0.1 gm./100 gm. in plasma protein concentration, and equates the effects of rise in arterial pressure resulting from occlusion of the carotid arteries with those of infusion of saline when they produce equal increases in sodium ouput. On the assumption that the change in sodium excretion is solely attributable to change in GFR, he calculates, as means of observations on three animals, that a rise in arterial pressure of 43 mm. Hg produces the same effect on sodium output as a fall in colloid osmotic pressure corresponding to a fall in plasma protein concentration of 0.18 gm./100 gm., both producing their effects by raising the GFR by 2.1 ml./min. The important feature of this calculation, if valid, is, perhaps, that this change in GFR amounts only to about 4 per cent, which is too small to be detected by normal clearance measurements in circumstances such as pressure or dilution diuresis which cannot be regarded as optimal steady-state conditions for such procedures. From one of his curves it appears than at increase in GFR from 52 to 56 ml./min. (8 per cent) is accompanied by an increase in sodium output of from 0.14 to 0.47 m. eq. per min. (226 per cent). On this view, the sodium output would seem to be a much more sensitive indicator of change in GFR than that provided by inulin clearance.

### Glomerular Permeability

Wallenius (31) has published an extensive and careful account of the excretion of hydrolysis products of dextran with different molecular sizes and shapes in order to determine the filtration constants of the glomerular membrane. Among interesting physical, chemical and biological observations too numerous to summarize the following may be mentioned. The molecular weights of the largest dextran molecules detectable in a 24-hour specimen of urine averaged 37,800 in guinea pigs and 45,700 in rabbits, in neither of which was proteinuria apparent. In rats, which normally pass some protein into the urine, the corresponding molecular weight was 62,700. In normal human subjects the average was 46,400, ranging from 42,500 to

49,000. In some abnormal cases it ranged up to 57,000 in burns without apparent proteinuria. In diseases involving proteinuria the size of the largest dextran molecules which were just detectable in the urine increased roughly in parallel with the degree of proteinuria up to values around 100,000.

In the dog the excretion of dextran with the low molecular weight of 4,000 is proportional to its plasma concentration, and its plasma clearance is substantially the same as that of exogenous creatinine, the dextran-creatinine clearance ratio being $0.99 \pm 0.06$. Restriction of filtration of the larger molecules reduced the clearance ratios with molecular weights of 8,500, 12,500, 16,500, 20,500, 24,500, and 28,500 to 0.909, 0.717, 0.487, 0.287, 0.163, and 0.110 respectively. Anesthetics had no significant influence on any of these values. Following Pappenheimer's (32) mathematical treatment, Wallenius calculates from his own data that the pore radius of the dog glomerular membrane may range from 18 Å to 50 Å. He notes that plasma albumin (diffusion radius (35 Å) and hemoglobin (radius 31 Å) do not pass through the glomerular membrane as readily as dextran molecules of the same size, and suggests that this may be related to the electrical charge of proteins which restricts their passage more than that of the electrically neutral dextrans.

Somewhat similar results on two dogs have been obtained by Giebish *et al.* (33). With dextrans of molecular weights about 7,500, 10,600, 35,000, 51,700, and 91,300, they found dextran-creatinine clearance ratios respectively of 0.94, 0.90, 0.18, 0.042, and 0.012. These authors also concluded that dextran was probably not transferred across the tubule walls in significant amount. The histological appearance of dextran in tubule cells must, therefore, be regarded as of no quantitative significance in relation to the comparatively large amount passing down the lumen of the tubules (31).

Lambert & Grégoire (34) have investigated the effects of variation of GFR on the proportion of hemoglobin transferred across the glomerular membrane. They injected about 5 gm. hemoglobin intravenously into dogs and found a fall in creatinine and PAH clearances and in the filtration fraction. When the GFR had fallen the hemoglobin clearance had changed little, and therefore the hemoglobin concentration in the glomerular filtrate must have risen. Similar results follow the reduction in GFR due to epinephrine. The rise in concentration in the glomerular filtrate is attributed to the large proportion of hemoglobin which is transferred by diffusion, the concentration gradient across the membrane being large compared with that of small molecules which are transferred mostly by filtration. Applying Pappenheimer's (32) equations they calculate that under normal conditions three-quarters of the hemoglobin is transferred across the membrane by diffusion and one-quarter by filtration. A reduction in GFR to one-half should then raise the hemoglobin concentration in the glomerular filtrate from 4 to 7 per cent which agrees well with experimental results. The change in GFR was not accompanied by a significant change in the transfer maximum for hemoglobin reabsorption.

A preliminary report by Herold & Bräutigam (35) states that whereas 50 per cent of injected polyvinylpyrrolidone (Periston) is excreted in 6 hr. in healthy normal women, 75 per cent is excreted in the latter third of pregnancy, excretion being accompanied by marked diuresis. The molecular weight of the substance in given as 60,000, and 2 gm. are injected intravenously. The increased output in pregnancy is attributed mainly to increased permeability of the glomerular membrane, but clearance studies,are said to suggest that 40 per cent of filtered Periston is normally reabsorbed, only 20 per cent being reabsorbed in pregnancy. Detailed evidence will be awaited with interest.

Electron microscopic studies of the glomerular membrane promise to throw light both on the pore size and on the effective thickness of the membrane. Hall (36, 37) found pores of a radius of 50 Å, but Reid (38) and Mueller *et al.* (39) have not been able to detect the presence of pores.

Pappenheimer (40) has undertaken an analysis of glomerular permeability as a development of his methods of investigating capillary permeability in muscle (32). In the hind limb he had found that capillaries had pores with effective radii of about 40 Å occupying less than 0.2 per cent of the histological surface of the capillaries. The glomerular system in the dog kidney allows inulin to pass practically as freely as creatinine. The ratio of myoglobin to creatinine clearance is 0.75, and the corresponding ratios for egg albumin, hemoglobin, and serum albumin are taken respectively as 0.22, 0.03 to 0.10, and less than 0.01. Inserting the values for myoglobin and egg albumen into the equations he calculates a pore radius of 35 to 40 Å for the glomerular membrane. Taking the thickness of the *lamina densa* as $0.5 \times 10^{-4}$ cm. (37), he derives the total area of the pores as 8 cm.$^2$ per gm. kidney, and taking Vimtrup's value for the total surface area of the glomerular membrane this amounts to 8 per cent of the histological surface. Whereas the pore sizes in glomerular and muscle capillaries are, therefore, not very different, the proportion of the surface of the former occupied by the pores is over fortyfold that of the latter. The filtration coefficient of the glomerular membrane can be calculated as about 3.1 ml./min./mm. Hg per 100 gm. kidney (dog). Taking the average value of the glomerular filtration rate in the dog as 62 ml./min. per 100 gm. kidney, this yields a pressure fall of 20 mm. Hg across the glomerular membrane, needed to overcome viscous resistance. If the filtration fraction in the dog be taken as 0.3, and systemic plasma protein concentration as 6.5 per cent (O.P. = 23 mm. Hg), the protein concentration of the plasma leaving the glomerulus will be 9.5 per cent (O.P. = 40 mm. Hg). The arithmetic mean colloid osmotic pressure opposing glomerular filtration is, therefore, 32 mm. Hg. The total pressure fall across the glomerular membrane must thus be about 52 mm. Hg. In an attempt to estimate the ranges within which these values should lie, taking into account possible modifications in the underlying assumptions and errors in empirical data, the filtration coefficient is said to lie within the limits of 1.9 to 4.5

ml./min./mm. Hg per 100 gm. kidney, the effective pore radius 35 to 40 Å, the aggregate pore surface 500 to 1,000 cm.²/100 gm. kidney occupying from 5 to 10 per cent of the total histological surface. The total hydrostatic pressure across the glomerular membrane at a normal filtration rate should then lie between 45 and 65 mm. Hg.

## Hydrostatic Pressures in the Kidney

Glomerular capillary pressure in the mammal still eludes direct measurement, but the clarification of the phenomena of autoregulation discussed above involves setting an upper limit to its likely value. Autoregulation of GFR is fairly effective in the range of arterial pressures between 80 mm. Hg and 200 mm. Hg. The glomerular capillary pressure cannot be much higher than, say, 70 mm. Hg at the lower end of the range, and in view of the relatively small change to be expected in glomerular filtration rate it is unlikely to exceed about 80 mm. Hg at the upper end of the range. At a normal arterial pressure of, say, 120 mm. Hg the glomerular pressure should, therefore, lie not higher than about 75 mm. Hg. This value agrees well with earlier estimates (16) based on the relations between ureter, venous and arterial pressures at constant glomerular filtration rate. If Pappenheimer's (40) estimate of the pressure fall across the glomerular membrane, 45 to 65 mm. Hg, be averaged this would indicate a pressure in Bowman's capsule of 20 mm. Hg.

The pressure in the lumen of mammalian tubules has recently been measured for the first time by Wirz (41) who applied micropuncture technique to the rat kidney during alcohol-water diuresis. In the middle segments of the proximal convoluted tubules, which are the most accessible from the surface of the organ, he finds pressures ranging from 9 to 32 mm. Hg. (mean 14.8 mm. Hg). Micropuncture of a few distal tubules (42) which were accessible yielded pressures between 10 and 17 mm. Hg during the diuresis. When, however, the diuresis was inhibited with antidiuretic hormone the pressure in the distal tubules fell abruptly to little above atmospheric pressure without much concomitant change in the pressure in the proximal tubule. Wirz suggest that this could be explained if the proximal tubular pressure were maintained by compression of the thin portion of Henle's loop by the interstitial pressure. The distal tubular pressure, on the other hand, may be determined by viscous resistance to flow in the peripheral parts of the tubules and the collecting ducts which are, presumably, too rigid to be compressed by the ambient interstitial pressure. The pressure was also measured in the blood vessel (41) near the distal points in the *vasa efferentia* where they divide; here the mean pressure was 17.4 mm. Hg, not significantly different from that in the tubules. This indicates an unusually low pressure in the peritubular capillaries which promotes water reabsorption into the blood stream.

The pressures in the renal vein and some of its tributaries have been

studied by Swann *et al.* (95) by retrograde cannulation with a fine polythene tube up the renal vein and beyond. The rather surprising discovery was thus made that in the arcuate veins the pressure was about 25 mm. Hg, but withdrawal of the cannula so that its tip lay in the interlobar vein only a millimeter or so below its junction with the arcuate vein resulted in a sudden fall of pressure to about 7 mm. Hg. The venous obstruction revealed in this way is in part responsible for the large volume of blood normally present in the kidney, estimated at 22 per cent of its normal functioning volume by Swann *et al.* (44, 96). This work has been developed along histological lines by Koester *et al.* (97), who first review earlier work on the musculature and valve-like structures of the renal venous system and then describe structures to which they attribute the obstruction to blood flow mentioned above. In man and the dog they find two relevant structures: First, stenoses at the junction of the interlobular and arcuate veins or near that of the arcuate and interlobar veins, with plain muscle conspicuously present in man but not in the dog, are probably of the greater functional significance. Secondly, sinusoidal cushions located nearby consisting of connective tissue pierced by large thin-walled sinusoids. In man, but not in the dog, these cushions also contain plain muscle. Swann & Weaver (98) have described the volume changes in dog kidneys, measured oncometrically, when the arterial pressure is varied. If the volume of the organ is taken at 100 per cent at an arterial pressure of 70 mm. Hg it is said to fall to 78 per cent with no pressure, and to rise to 110 per cent progressively as the pressure rises to 200 mm. Hg. The significance of intrarenal pressure remains puzzling. Miles & de Wardener (43) measured, with a capacitance manometer, the pressures in hypodermic needles plunged into the renal substance, and found values ranging from 9 to 38 mm. Hg, mean 21 mm. Hg, in anesthetized dogs. The needle pressures were sensitive to variation in arterial pressure, reduced by vasoconstrictors and increased by osmotic diuretics, confirming results of earlier workers, particularly those of Swann (44). Decapsulation has no effect even after administration of potassium cyanide, which latter could raise the needle pressure to 100 mm. Hg at a perfusion pressure of 300 mm. Hg. In mannitol diuresis the needle pressures rose, averaging 36.2 mm. Hg. These authors, like Swann and others, regard the pressure measured in such needles as representative of the intrarenal pressure, the term being used in the same sense as used by Winton (18), i.e. a pressure in renal tissue capable of compressing the tubules and thus obstructing the outflow of urine. The original method of assessing intrarenal pressure in terms of the least ureter pressure which will reduce the urine flow gives however much lower values in the anesthetized dog (16), and even in swollen edematous perfused kidneys its value averaged only 10 mm. Hg; it then being insensitive to variation in arterial pressure, but reduced by decapsulation, and increased in diuresis of all kinds.

If our earlier assessment of the pressure in Bowman's capsule is about

right at 20 mm. Hg, it clearly would not be possible to suppose that the pressure at the peripheral end of the tubule was 26 mm. Hg as a result of intrarenal pressure as suggested by Swann (44). If so, it would have to be even higher than 26 mm. in osmotic diuresis. The wide variation in needle pressure found in different kidneys and in different parts of the same kidney suggests the possibility that intrarenal pressure in the normal kidney may be no higher, say 2 to 4 mm. Hg, than interstitial pressure in other tissues, but that rupture of blood vessels and other tissues by the hypodermic needle may create as an artefact a very small pool of liquid round the end of the needle, deriving its pressure as a resultant of a supply of fluid from ruptured arterioles and a leak of the fluid to ruptured venules and tubules. This leak would be much less in the kidney than in other tissues such as muscle or skin because the substance of the renal tissue is much denser and more rigid, hence the relatively higher values of needle pressure found in the kidney.

## Structural Considerations

There are two methods of enumerating the glomeruli in the dog's kidney. Earlier workers who had employed computation from histological sections have found a strikingly lower value than those who depended on maceration of the kidney. Sellwood & Verney (45) examined both methods, the histological method being immensely time-consuming because of the uneven distribution of glomeruli. In a single kidney from a dog, 19.8 kg., they calculate that 653,000 glomeruli are present according to their histological technique, and 630,000 according to their maceration technique. Hartman (46) has studied the phenomenon of intermittence in glomeruli rendered visible by translumination of the kidney in the immature anesthetized mouse. The best age for the purpose is 10 days, but only the glomeruli containing red cells and sited near the surface could be seen. Capillary intermittence involving the presence of cells in some loops only of a glomerulus was usually seen, but total glomerular intermittence occurred only under abnormal conditions, such as intravascular agglutination. The capillary intermittence may, it is suggested, constitute a physiological reserve, but it may occur only in the immature animal. Glomerular intermittence, however, was indicated fairly commonly in rats after injection of India ink by Still & Whitcomb (47), who inserted a fine polythene tube into the aorta of rats and brought the other end of the tube out through the skin at the back of the neck. Three to five days later they injected India ink for 5 sec. and immediately guillotined the animal between the heart and kidney. The kidney was fixed in 10 per cent formalin, and celloidin sections cut with a view to identifying shunts. In 9 out of 13 controls all blood vessels appeared full, i.e. there were no shunts patent which would lead to emptying or inaccessibility to the ink of the glomerular vessels. Adrenalectomy, exercise, vasodilators and administration of water had no effect. Large doses of epinephrine and vasopressin (Pitressin) closed most of the vessels distal to

the interlobular vessels. Injection of 2 per cent saline produced indication of shunts in about one-half the animals. Of the seven types of vascular shunt which have been described in the literature, the authors saw evidence of only two, the juxtamedullary glomeruli and the arterialrecti verae running from the arcuate artery to the peritubular plexus.

Localization of function in the tubule of the rat kidney has been advanced by micropuncture experiments of the middle third of the proximal convoluted tubule. Wirz & Bott (48) have found that the concentration ratios of tubular fluid to plasma vary from 0.26 to 1.14 for potassium and from 0.30 to 0.76 for reducing substances. Neither ratio is obviously related to the urine/plasma concentration ratio. Potassium and glucose are, thus, reabsorbed at about the same site, but the mechanisms must be different because the variations in the ratios do not correspond.

The secretory functions of the pronephros and mesonephros of the larval frog have been compared by Jaffee (49) employing the methods developed by Forster & Taggart for the flounder kidney. Phenol red begins to be concentrated on the ninth day and is adequately transferred by the pronephros tubule by the eleventh day (11 mm. tadpole). The pronephros persists for most of three months. Meanwhile the mesonephros has become functionally complete at the end of the first month when its tubules begin to transport phenol red. Hence the pronephros and mesonephros function together for two months. The transport of the dye requires provision of oxygen and is inhibited by cyanide or sodium azide in concentrations which are the same for the pronephros and mesonephros, indicating inhibition of the cytochrome oxidase system. Oxidative phosphorylation and nonoxidative phosphorylation are said to be inhibited by 2,4-dinitrophenol, and by trinitrophenol respectively, and both of these are equally effective in the two organs. Anerobic glycolysis and competitive inhibition by PAH, penicillin, and uric acid have also been studied. Comparatively high concentrations of creatinine have no effect on phenol red transport; thus creatinine appears not to be secreted by either pronephros or mesonephros in the frog, which show in all these respects a close functional resemblance both qualitatively and quantitatively.

## The Measurement of Renal Plasma Flow and Glomerular Filtration Rate

Sellwood & Verney (12) have re-examined the values for renal plasma flow (RPF) in the heart-lung-kidney preparation by comparing direct measurement of blood flow and hematocrit with the Fick principle applied to PAH. They estimated PAH concentrations in arterial cells and plasma and in venous cells and plasma and found the content in venous cells substantially higher than that of arterial cells. Hence, in this preparation, the extraction ratio is too high, and the derived plasma flow too low. Extraction ratios in the experiment quoted ranged from 0.77 to 0.85. In the unanesthe-

tized animal the PAH content of cells was also a little higher in the venous than arterial blood. The equilibrium between plasma and cells was established with constant infusion of PAH within half an hour. With infusion of creatinine equilibrium between cells and plasma had not been reached in two hours. Nevertheless the authors are reasonably satisfied with the usual methods of estimating RPF and GFR.

Hare & Hare (50) remark on the absence of histochemical evidence respecting tubular secretion or reabsorption of inulin or creatinine contrasted with the presence of such evidence confining ferrocyanide to filtration. Earlier work comparing ferrocyanide, creatinine, and inulin clearance ratios had been performed after a single intravenous injection with correspondingly insecure results. These authors have repeated the comparisons in nine unanesthetized dogs during constant intravenous infusion of the substances concerned and have made simultaneous clearance measurements. The ratios correspond very closely to unity both at high and at low plasma concentrations, providing further validation of inulin and creatinine clearances as measures of glomerular filtration rate. The systematic difference between ferrocyanide and exogenous creatinine clearances, corresponding to the ratio 0.966 given by Berliner *et al.* (51), was not found.

## The Pituitary Gland and the Kidney

Abrahams & Pickford (52) have investigated the effects of agents with an antidiuretic action to discover whether they would simultaneously increase uterine activity resulting from liberation of oxytocin. The experiments were performed on unanesthetized dogs with a balloon in one uterine horn. The stimuli applied included intracarotid or intravenous injections of hypertonic saline, sucrose, acetylcholine, and procedures expected to arouse emotion. All these agents were found to increase uterine activity for about the same length of time as they produced antidiuresis. Hypophysectomy resulting in diabetes insipidus prevented the antidiuretic response to hypertonic saline which, however, still caused some increase in uterine activity, but this differed in its time course from that seen in normal animals. Reasons are given for concluding that the increase in uterine activity in normal dogs is attributable to liberation of oxytocin, but in order to simulate the antidiuretic and uterine responses which simultaneously follow appropriate stimulation of pituitary secretion it was necessary to inject very different amounts of the two principles, less than 5 mU of vasopressin contrasted with 75 to 100 mU of oxytocin. Since the potency of both pituitary principles is, by definition, equal in an extract of the gland, this would suggest that there are two distinct active substances which can be liberated at unequal rates. This is an important further contribution to the long controversy between those who believe that a single substance is secreted by the neurohypophysis and those who believe there are at least two such substances.

The depression of renal function after neurohypophysectomy has been

studied by Demunbrun *et al.* (53) in order to discover whether it should be attributed to lack of the secretion of the adenohypophysis or to lack of oxytocin. In eleven unanesthetized dogs, the RPF, GFR, and $Tm_{PAH}$ after hypophysectomy were only 50 to 60 per cent of what they had been before the operation. The operated animals showed no signs of anterior pituitary deficiency. Both Pituitrin and oxytocin (Pitocin) injections restored, wholly or partially, the depressed renal functions, a development which was independent of change in arterial pressure. Clearly, therefore, the depression was attributable to deficiency of oxytocin. It is intriguingly proposed that this may indicate the normal physiological function of oxytocin in the male.

The problem whether a hypertonic urine can be produced by methods not involving the action of the antidiuretic hormone has engaged Leaf *et al.* (54). In dogs the right kidney was explanted under the skin and a cuff placed round the right renal artery. Both ureters were explanted so that the output from the normal left kidney which served as control could be compared with that of the right kidney when the renal artery was partially occluded in the unanestheized animal. In some experiments the urine was dilute because of water diuresis; in others it was more concentrated as a result of dehydration and glucose infusion. Compression of the renal artery reduced the GFR relatively more than the RPF but failed to produce a concentrated urine. During water diuresis the urine flow varies with the GFR under the influence of compression, but there is little change in its solute concentration. In glucose diuresis the mechanical lowering of the GFR may even result in a lowering of urinary solute concentration, presumably attributable to loss of concentrating power when the blood supply is inadequate. Concentration of the urine can, it is believed, be produced only by the antidiuretic hormone.

The liberation of antidiuretic hormone during muscular exercise in man is indicated by observations of Croxatto *et al.* (55). Human venous blood drawn before breakfast and injected intraperitoneally into hydrated rats usually produces some antidiuresis, but a more powerful antidiuretic action of the blood is shown after 30 min. excerise on a bicycle ergometer. If 650 ml. water had been administered at the beginning of the half-hour exercise period no antidiuretic activity could be detected. The interpretation of antidiuresis after injection of blood into rats is, however, unfortunately, not without difficulty.

Thiosorbitol has, it is claimed, the capacity of inactivating the oxytocic action of oxytocin and the vasopressor action of vasopressin without inactivating the antidiuretic action of vasopressin. This phenomenon, applied to very large doses of vasopressin, has received further study by Croxatto *et al.* (56), in the hope of deciding whether the vasopressor and antidiuretic activities are attributable to two distinct substances or involve different chemical groups in a single substance. The authors incline to the latter view.

## Theoretical Considerations

A thermodynamic analysis of the intracellular osmotic gradient hypothesis of active water transport has been undertaken by Brodsky *et al.* (57) to test the validity of the Franck-Mayer theory. The minimum rate of expenditure of free energy is calculated from representative dimensions of tubule cells and concentrations of solutes during the formation of a hypertonic urine. The value obtained for unit volume of cells is 21,000 kcal. per kg. per hr. This is stated to be about one thousandfold the maximum measured metabolic rate of living cells, and the theory from which it is derived is, therefore regarded as untenable. Wesson (58) has developed the mathematical analysis of urea excretion in the dog based on Shannon's theory. This explained the facts that in water diuresis the urea/inulin clearance ratio approaches about 0.70 asymptotically as the inulin U/P ratio approaches unity, but that in osmotic diuresis the urea-inulin clearance ratio approaches 1.0. Two tubular segments are involved, the distal segment which is relatively impermeable to urea and the proximal segment which is more permeable and consequently is affected by osmotic agents. Among the assumptions on which the equations for each segment are based it is supposed (*a*) that urea transfer across the tubule wall is proportional to the concentration gradient, (*b*) that the urea concentration in the peritubular fluid is uniform and equal to that in the glomerular filtrate, (*c*) that three segments of tubule can be distinguished, a proximal one with moderate permeability, a distal one with low permeability and the collecting system which is impermeable, (*d*) that the permeability of a given segment is taken as constant, there being no interdependance of proximal and distal segments, (*e*) that the entire kidney is taken as a multiple of a single nephron, and (*f*) that the water reabsorption in a given segment is postulated as the same per unit length of tubule. This last postulate seems at variance with the presumed osmotic equilibrium between the fluid in the proximal tubule and the peritubular interstitial fluid near it. Moreover it is also assumed that osmotic diuretics reduce the total loss of water from the proximal tubules. If it had been assumed that the rate of loss of water were such as always to preserve osmotic equilibrium at each particular point along the tubule, the resulting equations would no doubt have been much more complicated and probably very difficult to handle.

The author shows that if his simplifying assumptions are accepted the data obtained by Shannon on urea/inulin clearance ratios can be accounted for reasonably well both in water diuresis and in osmotic diuresis. The loss of urea by simple diffusion from the tubules would seem to be some 25 to 30 times greater in the proximal than in the distal tubules. This analysis of tubular function probably constitutes an important advance in the precision of understanding the processes concerned. The presentation is, however, somewhat obscure and condensed, in part perhaps attributable to the edito-

rial demand for brevity, and one must await a more extended and definitive publication before its value can be safely assessed.

As an addendum on urea excretion may be mentioned Forster's (59) interesting analysis of the active transport of urea by the frog's renal tubules. Active transport of urea is rare in vertebrates, but in the frog its clearance may be four to ten times the GFR though only one-third of the PAH clearance. A transport maximum for urea was reached when its concentration in plasma exceeded 15 mg. per 100 ml., and values for the Tm ranged in bullfrogs from 6 to 20 mg./kg./hr. in different animals. When aerobic phosphorylation is blocked by 2,4-dinitrophenol the active transport of urea is prevented and some passive reapsorption can often be detected. PAH can reduce the active transport of urea by competitive inhibition by 50 per cent or more. The author points out an important advantage to the frog in eliminating urea actively, in that the GFR may be very low when the animal is dehydrated but urea may still be eliminated by tubular secretion since the tubules are supplied with renal portal blood.

### Electrolyte Excretion

The renal excretion of chloride in unanesthetized and lightly anesthetized dogs has received comprehensive study by Wesson & Anslow (60) who investigated its relation to GFR, plasma chloride and bicarbonate concentrations, and urinary osmotic load. When the GFR was varied by infusion, bleeding, or restoration of blood, the chloride reabsorption was found to be a linear function of the GFR but unaffected by changes in concentration of chloride in the plasma. In urea or mannitol diuresis the reabsorption is less, and the slope of the curve relating reabsorption to glomerular filtration rate is lower. Ions, such as bicarbonate, also produce a lowering of chloride reabsorption, in so far as they act as osmotic diuretics, but in addition there is further reduction in reabsorption as a result of competition with the chloride ion in the reabsorption process. The reabsorption of chloride, like that of sodium is thought to be attributable to two independent processes: (*a*) a "proximal" process involving reabsorption of large amounts, proportional to the glomerular filtration rate and reduced by osmotic diuretics, and (*b*) a "distal" or facultative process, subject to a transport maximum which is reached in a maximal water diuresis.

Greene & Hiatt (61) on the contrary claim that only nitrate and thiocyanate have a greater chloruretic effect for a given urine flow than mannitol. In unanesthetized dogs they find nitrate itself is reabsorbed at low plasma concentrations but when this exceeds 20 m. eq./l. the excreted nitrate remains fairly constant at 30 to 40 per cent of that filtered. It increases the chloride excretion to 5 to 10 per cent of that in the filtrate and may increase sodium excretion up to 15 per cent of that filtered. Injected nitrate has no effect on arterial pressure but increases the GFR measured as exogenous creatinine

clearance during continuous infusion of creatinine. This rise in GFR is said not to be accompanied by a corresponding rise in reabsorption of sodium, chloride, or nitrate.

The factors concerned in the excretion of sodium and chloride in the unanesthetized dog have also been examined by O'Connor (29). Denervation of the kidneys and administration of deoxycorticosterone had no effect on the excretion of a dose of 2.5 to 3.5 gm. of NaCl. The plasma sodium and chloride concentrations do not correlate with the amounts excreted. However both dilution of plasma proteins and increase in arterial pressure induced by carotid occlusion increase the output of water, sodium, and chloride as mentioned in the section above on autoregulation. Ingestion of ammonium chloride or water caused no increase in sodium excretion and little change in plasma solids. Whereas ingestion of sodium bicarbonate increased the excretion of sodium but [in contrast with Wesson & Anslow (60)] not that of chloride, it produced a dilution of plasma solids. The author emphasizes the significance of small changes in GFR, too small to detect by the usual clearance methods, in production of relatively large changes in sodium and chloride output. For example, an increase of GFR estimated at 1 ml./min., i.e. about 2 per cent, increased filtered sodium by 0.153 m. eq./min., and of this 0.095 m. eq./min. or 60 per cent appeared in the urine. Unfortunately the author's way of estimating glomerular filtration rate is at least as indirect and open to error as are the clearance methods. It involves determination of the solids in as little as 0.3 ml. plasma by drying at 95° to 105°C., there being apparently no further weight loss after 3 hr. From this weight were subtracted quantities at the rate of 0.9 gm. per 100 gm. plasma for sodium salts and 1.0 gm. per 100 gm. plasma for solids other than proteins, and what remained was taken as representing plasma proteins. Changes in this protein content were translated into changes in GFR by assuming the values found by Wesson *et al.* (30)

In anesthetized dogs a small hemorrhage, producing a fall in arterial pressure less than 20 mm. Hg, produces a fall in urine flow and output of sodium and chloride, according to Goodyer & Jaeger (62), even when there are no detectable reductions in inulin or PAH clearances. Hexamethonium in a dose which produces the same fall in pressure produces no consistent effects on urinary output, which is believed, therefore, to depend on blood volume and not on blood pressure. Re-infusion of the shed blood, after a hemorrhage, restored the renal function concerned with water, sodium, and chloride output even after autonomic block or denervation of the kidney, and when arterial pressure, RPF, and GFR had undergone only insignificant changes. These authors, like O'Connor, regard hormonal influences on tubular reabsorption as inapplicable to these responses because they occur too quickly.

Intravenous injection of isotonic or hypotonic mannitol solutions in man were also found by Williams *et al.* (63) to produce diuresis involving increased

output of sodium and chloride. The sodium excretion peaked at three to five times the pre-mannitol values. The increase in electrolyte output occurred even when the GFR, measured as endogenous creatinine output, declined, and when neither the water load in the body, the extracellular fluid volume, nor the plasma molarity had changed. If all these conclusions were fully justified the authors' conclusion that mannitol acts directly on the tubules would be inescapable.

Experimental tricuspid valve insufficiency in dogs, which has been found by Barger *et al.* (64) to increase the right atrial pressure to 10 to 14 cm. water, produces a moderate reduction in renal sodium output with no reduction in urine flow. Exercise increases the sodium excretion in normal dogs but not in tricuspid-deficient dogs. Congestive failure may reduce the output of sodium to 1 to 2 per cent of an infused load without much reduction in water output. Hence the primary effect is on the sodium reabsorption. The authors believe that neither rise in venous pressure nor diminution in cardiac output are essential causes of the failure to eliminate sodium but they cannot decide what is the essential cause. Howell & Davis (65) have studied the relationship of sodium retention to potassium excretion in unanesthetized dogs during administration of deoxycorticosterone acetate (DCA). It is known that in man, the dog, and the rat, DCA increases potassium excretion, reduces potassium concentration in the plasma, and produces metabolic alkalosis, all of which effects vanish in the absence of an adequate intake. Moreover dogs with a partial constriction of the thoracic inferior vena cava exhibit sodium retention. Administration of DCA to dogs with the constriction does not affect the sodium retention, but it now does not produce the changes in potassium metabolism even when the sodium intake is adequate. Adrenalectomy produced no significant changes in these responses. The failure of the potassium excretion to increase in normal dogs in the absence of sufficient sodium intake might, it is suggested, result from the almost complete reabsorption of sodium and chloride if it were supposed that the potassium excretion depends on a sodium-potassium ion exchange system distal to this reabsorption.

### Diuretics and Other Drugs

120 cc. of proof bourbon (48 gm. ethyl alcohol) was drunk neat by each of three normal males during 5 to 10 min., a celebration which might perhaps be expected to evoke some circulatory or other nonspecific response. Controls imbibed 120 cc. water. Rubini *et al.* (66) compared the creatinine, sodium, potassium, ammonium, and chloride excretion, the pH of the urine and its acid content in these subjects in a study of alcohol diuresis. The plasma alcohol concentrations rose for 60 to 90 min. with a peak value averaging 0.11 per cent. The concentration then declined in a time-course representing an exponential curve, rather surprisingly, since most previous observers have described an approximately linear decline in concentration after such

comparatively low peak blood concentrations. In the present study the diuresis proceeded without change in GFR, measured as creatinine output, but with a decreased solute excretion. There was also acidosis involving increased ammonia and titratable acid in the urine. The fall in output of sodium, potassium, and chloride has been noted by others but its cause is not known. In a further study of alcohol diuresis induced in the same way the same authors, Kleeman *et al.* (67), attempted to define more precisely the degree to which ethyl alcohol can inhibit the secretion of the antidiuretic hormone. Bourbon had no further diuretic action at the height of a water diuresis, during which pituitary activity was presumably minimal. Bourbon blocked the antidiuretic effect of hypertonic saline and minimized the antidiuretic effect of venous congestion of limbs, both conditions being associated with enhanced pituitary secretion. The alcohol diuresis itself was, however, prevented by a combination of the results of previous administration of hypertonic saline and venous congestion of limbs. These observations confirm the view that alcohol is a powerful inhibitor of the supra-optico-hypophysial mechanism controlling secretion of the antidiuretic hormone, but not, in the dose given, necessarily a pre-potent inhibitor.

Drastic hydration was induced in normal and hypertensive men by Ek (68) who infused 3 to 5 per cent glucose solutions intravenously at 25 to 30 ml./min. and produced diuresis varying from 3 to 30 ml./min. In normals, the inulin clearance rose from 130 to 160 ml./min. but all the increased filtered sodium was reabsorbed, the output remaining at 400 $\mu$ eq./min. The PAH clearance also increased slightly from 800 to 850 ml./min. In hypertensives there was proportionately a greater rise in the lowered inulin clearance from 70 to 95 ml./min. and also of the PAH clearance from 350 to 500 ml./min. In them, the sodium excretion was greatly increased from 250 to 700 $\mu$ eq./min. Drinking two liters of beer or water also increased sodium output in hypertensives, a useful clinical test of functional deterioration. Theophylline administered during the diuresis resulting from intravenous infusion produced a large but rather short-lived increase in the diuresis, say from 30 to 50 ml./min. with an increase in GFR but little or no change in RPF. The sodium output, however, then increased both in normals and in hypertensives. Theoretically interesting is the possibility of raising the GFR substantially in normal men by hydration without increasing the sodium output.

GFR could be raised to 85 ml./min. in unanesthetized dogs on a pork heart diet, but only to 66 ml./min. on a casein diet calculated to have an equal protein content, according to Tank & Herrin (69). Neither produced proteinuria. They found that intravenous injection of alanine increased GFR about 30 per cent and RPF about 40 per cent while after intravenous injection of valine there was usually no change in these variables. The mechanisms of the responses remain mysterious.

Mercurial diuretics are believed by Farah & Koda (70) to have independ-

ent actions on the urinary water and sodium outputs in both anesthetized and unanesthetized dogs. It was previously known that mercury compounds usually induce a rise in sodium excretion which precedes the rise in water output. In animals with a low initial sodium concentration in the urine produced by 3 per cent glucose infusion at 0.5 ml./min. per sq. m. for 2 to 3 hr. a small dose of mersalyl, say 1 mg., increases the sodium excretion without affecting water excretion, while a larger dose, say 10 mg., increases both without affecting the GFR. The effects are partially reversible with small doses of 2,3-dimercaptopropanol (BAL) which reverse the increase in urine flow but may further increase the output of sodium.

A four-point diuretic bioassay of mercurial and xanthine diuretics on patients with congestive heart failure has been developed by Greiner *et al.* (71) in terms of the weight lost 24 hr. after administration of the dose. The absolute weight losses observed were of the order of 1 pound, rather alarmingly small. Injected intramuscularly, meralluride (Mercuhydrin) showed about tenfold the potency of aminophylline, the doses being expressed in molar terms. Administered by mouth, aminophylline was only a little less effective than meralluride when they are compared by their effects in producing a small diuresis; the maximal diuresis which can be produced by larger doses of aminophylline is, however, much smaller than the maximal which can be produced by meralluride. Aminophylline and calcium theophyllinate were equally active in equimolar doses but the toxic effects of these large doses of xanthine diuretics were more prominent than those for the best mercurial diuretics. The diuresis produced by meralluride can be increased 50 per cent or so by aminophylline; this is a purely additive action, not a potentiation such as that produced by ammonium chloride. No evidence was found that xanthine diuretics, with a mainly vascular action, were effective in cases resistant to mercurial diuretics, with a tubular action.

All anesthetics are believed by Papper (72) to produce a nonspecific deterioration in renal functions which, according to evidence in this review article, is unaffected by denervation or ganglion-blocking agents but might be the result of circulation of increased amounts of epinephrine or norepinephrine. In careful studies by de Wardener (73) of the effects of ether and cyclopropane, on the other hand, their action in increasing the resistance of the renal vasculature in dogs is said to be abolished by denervation, a conclusion which accords with that of many earlier observers. Light anesthesia reduced the renal blood flow (PAH clearance and venous hematocrit) in these experiments by 32 per cent and deep anesthesia by 63 per cent, about equally for ether and cyclopropane and independently of the duration of their action. Fairly drastic surgical procedures usually had no further effect on the renal blood flow. Pentobarbital is described as having little effect on the renal circulation. Hypertensive drugs give variable results. For example a 64 per cent rise in arterial pressure produced by norepinephrine reduces the renal blood flow by 18 per cent, while the same rise in pressure produced by

*d*-deoxyephedrine hydrochloride (Methedrine) increases the flow by about 20 per cent. An 11 per cent rise in arterial pressure produced by epinephrine reduces the flow by about 18 per cent. Hypotensive drugs had earlier been shown to have little effect on the flow; for example, pentamethonium bromide which reduced arterial pressure 34 per cent reduced the renal blood flow only 5 per cent. Blood loss not greater than about 23 per cent of the blood volume, when performed under light ether or cyclopropane anesthesia, produced little immediate effect on either arterial pressure or renal blood flow.

The effects of pentobarbital and chloralose anesthesia, after intravenous injection, have been studied by West & Keller (74) during mannitol diuresis. Only pentobarbital produced a transient fall in GFR. In hydrated animals it reduced the urine flow and increased its osmolarity and sodium content, while chloralose if anything reduced the sodium concentration. In hydropenic animals pentobarbital reduced the sodium output transiently, but this was followed by a rise above the normal value. The effects of the anesthetics on sodium excretion were the same after denervation of the kidneys, and are not, therefore, related to sympathetic activity; the possibility of a humoral mechanism is considered.

A direct sympathetic activity on the tubules also seems to be excluded by the experiments of Handley & Moyer (75) on anesthetized dogs and on human patients. Norepinephrine in doses which raised arterial pressure to 170 mm. Hg or less produced diuresis with a rise in GFR but a fall in RPF. Doses large enough to raise the pressure above 170 mm. Hg reduce all three variables. The sodium excretion follows the GFR, and there is no evidence of any action of the drug directly on the tubules. The fall in arterial pressure resulting from hexamethonium produced no consistent changes in RPF or GFR, whereas a renal vasodilator (N substituted arterenol) raised arterial pressure and the RPF without effect on the GFR. None of these drugs had any specific action on glucose Tm or on sodium reabsorption, any observed changes being related to GFR changes. These authors have extended their observations on pentobarbital-anesthetized dogs to define the effects of adrenergic blocking agents on renal function particularly in view of the suggestion that Hydergine diuresis was due to inhibition of sympathetic activity, although this drug also acts on the central nervous system. Thus Moyer *et al.* (76) found little change in arterial pressure or $Tm_G$ and a slight rise only in GFR after administration of Hydergine, but the urine flow increased 80 per cent, the sodium output 178 per cent, and the potassium output 63 per cent. Phenoxybenzamine produced about 40 per cent fall in arterial pressure and a 7 to 13 per cent fall in GFR with hardly any change in urine flow or electrolyte excretion. Phentolamine had somewhat similar actions, while hydralazine reduced arterial pressure, urine flow, and sodium output with no change in potassium output, but the latter drug also has actions on the central nervous system. The authors conclude that adrenergic

blocking agents have little effect on tubular activity and that the diuretic action of Hydergine is attributable to an unknown action on the central nervous system.

Renin has been found by Rosenfeld *et al.* (77) to have little effect on the transport maximum for PAH in the anesthetized rabbit although it produces diuresis and reduced reabsorption of sodium and chloride and probably of protein. Renin also produces no effect on creatinine clearance, but the authors feel unsure whether creatinine clearance is a safe measure of GFR in the rabbit in spite of earlier reports of its being equal to the inulin clearance, because the creatinine clearance can be reduced, maybe to one-half, by increase in PAH concentrations in the plasma. This may well be the result of vasomotor effects with a consequent fall in GFR, but the authors regard tubular secretion of creatinine and competitive inhibition by PAH as a serious possibility.

### Miscellaneous

Do agents which lower arterial pressure reduce glomerular filtration rate more or less equally in all nephrons or shut down some nephrons altogether? The question is raised by Handley & Moyer (78) in a reinvestigation of the much-disputed relation between glomerular filtration rate and $Tm_G$, both in anesthetized dogs and in human patients. They found that the ratio of GFR to $Tm_G$ was fairly constant when the GFR was reduced by infusions of epinephrine or norepinephrine or by doses of morphine, and they concluded that a variable proportion of nephrons become completely ischemic under those conditions. These authors do not here compare the effects of drugs, in reducing GFR, with those of mechanical reduction of renal arterial pressure which has been thought to produce little change in $Tm_G$ in spite of a fall in GFR.

An interesting paper on the effects on renal function in the bullfrog of dehydration and low temperature has been published by Schmidt-Nielsen & Forster (79). The paper (59) on a cognate field, mentioned earlier, adds to its value. It is known that frogs immersed in water gain water through the skin four to five times as quickly at 30°C. as they do at 0°C., and, since the kidneys eliminate water at a rate which keeps the body weight constant, the urine flow varies correspondingly with temperature. In air at room temperature frogs lose about 25 per cent of their body weight in 10 hr. by evaporation. In these circumstances the GFR falls to about 50 per cent in 2 hr. and 15 per cent in 5 hr. with reduction in urine flow. Pituitary injection also reduces both the urine flow and GFR but it has no effect on tubular activity. In cold water the body weight rises slowly with fall in plasma osmolarity from 110 m*M* to 90 m*M*. Later, urine flow slowly develops, with the GFR perhaps one-seventh normal and tubular reabsorption of water very low. Average values in normal circumstances of RPF (PAH clearance) were 775 ml./kg./hr., and of GFR (creatinine clearance) 30.4 ml./kg./hr. The filtra-

tion fraction is fairly constant at 0.04 varying only 20 per cent when the GFR varies from 3 to 57 ml./kg./hr. $Tm_{PAH}$ and $Tm_G$ are proportional to the GFR indicating intermittent activity of the nephrons. The average urea/creatinine clearance ratio is $6.9 \pm 0.3$ but the average PAH/urea clearance ratio is $3.4 \pm 0.2$. This comparatively low secretion rate for urea may be because the plasma urea concentration exceeds the saturation point of the active transport mechanism, but some urea may also be lost by diffusion from the tubules.

The active transport of phenolsulfonphthalein and related dyes has been examined in isolated tubules of the flounder and in kidney slices of dogfish by Forster *et al.* (80). As in the chicken, phenol red is transported most rapidly, bromcresol green the most slowly, and five other dyes intermediately. Those dyes which are transported slowly are the most powerful in competitive inhibition. Bromcresol green is a potent inhibitor not only of chlorphenol red but also of PAH.

Renal function in the first two days of life has been compared with adult function in humans by McCance & Widdowson (81). Developing their important and unusual conception that renal function should be assessed quantitatively not according to body weight or to surface area but according to the volume of body water, they find that the babies secreted urine at a rate of 13.8 ml./l. body water per day, the corresponding figure for adults being 16.8 ml./l. per day. Solute concentration in infants was only 420 m. osmol./l. against adults with 1,224 m. osmol./l. In infants urea clearance increases with urine flow, averaging 9.1 ml. per 42 l. body water/min. on the first day, 14.6 ml. per 42 l./min. on the second day, and 37.2 ml. per 42 l./min. in adults. After prolonged or difficult labour they find (82) a lower urea clearance and, presumably, GFR than in normal infants, an increase in nitrogenous excretion and a large excretion of phosphorus during and just after birth, contrasted with the very low phosphorous excretion of normal infants in their first day. Koletsky (83) has followed the changes in rats' kidneys after arrest of the renal circulation for periods of various duration. Necrosis is always confined to the proximal convoluted tubules, the degree depending on the duration of ischemia. The damage is fully developed in three weeks, and remains indefinitely unless the normal kidney is removed. If it is removed three weeks after the injury complete compensatory hypertrophy and restoration of adequate function follows if ischemia of the injured kidney had been confined to 2 hr. If ischemia had lasted 4 hr. or more the injury is irreversible.

Fluorescein excretion in man has been compared with creatinine and PAH clearances by Schwalb *et al.* (84). Fluorescein clearance is said to be little higher than that of endogenous creatinine, but these observations were made after a single intravenous injection of this highly diffusible substance with the clearance values based on the content of venous blood, and they presumably have only limited validity. Excretion begins 3 min. after the

injection, the urine flow being brisk after drinking 500 ml. water. Excretion is almost complete in 2½ hr. and only traces can be detected after 7½ hr.

Penicillin clearance as a renal function test has been examined by Pers (85) both with and without collection of urine, with special reference to its application to patients with, for example, ureto-colic anastomosis in whom urine cannot be collected. Penicillin is particularly suited to constant infusion technique because, whereas the range of plasma concentration so achieved varies very widely with the magnitude of the unknown clearance, the range from the smallest concentration of penicillin at which estimation in plasma is fairly accurate to the concentration where self-depression of clearance begins is very wide—1:100 for penicillin compared with 1:10 for PAH or Diodrast. After a priming dose a constant infusion of 1,000 U/min. is given for 5 hr. and achieves a concentration in plasma of 150 U/ml. when the clearance is 75 ml./min. and 1 U/ml. when the clearance is 1,100 ml./min. Clearances less than 75 ml./min. prevent achievement of steady plasma concentration in 5 hr. Clearances calculated indirectly from the infusion rate agree well with those obtained from the urine content during steady plasma concentrations, the standard deviation for both methods being about 10 per cent.

A considerable number of workers have continued the investigation of various aspects of acid and alkali control by the kidney which are included in the review by Pitts (86) on the general problem of tubular activity. The effects of acidosis and alkalosis have been discussed *inter alia* by Brodsky (87), Jenson *et al.* (88), Sullivan & Dorman (89), and Holliday (90). The factors concerned in ammonia excretion have been analyzed by Rector *et al.* (91, 92), and those concerned with $CO_2$ tension in urine by Dorman *et al.* (93). The effect of administration of a carbonic anhydrase inhibitor in renal disease in man has been studied by Kaye (94).

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# LIVER[1]

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Among many important developments in this field, this reviewer finds most promising the increasing trend towards correlating information concerning liver structure, liver function, and perhaps liver injury. Since selection of topics for consideration here was necessitated by the plethora of published material,[3] the reviewer has given precedence to work illustrating this development, touching lightly upon a few other important areas, and for the rest entrusting the reader to some excellent recent reviews and discussions.

## The Fluid Compartments of the Liver

*Osmotic relations.*—Swelling, sodium content, potassium content, and $Q_{O_2}$ of liver, kidney, and brain slices incubated in media of varying electrolyte composition, colloid content, and temperature (1, 2) provide evidence of two separate mechanisms controlling tissue water intake and electrolyte composition. In the liver, as in kidney, these are readily impaired at low incubation temperatures, resulting in reversible swelling, potassium loss, and sodium uptake. The author concludes that liver cell contents *in vivo* are hypertonic to blood plasma; that, in addition to the above mechanisms, tissue tension facilitates maintenance of this state.

Freezing points of frozen thawed liver suspensions were not found to be different from blood serum (3). On the other hand, in slowly frozen livers, ice crystal formation occurs first in the extracellular phase (4); on thawing and refreezing, this difference between cells and extracellular phase vanishes.

Warming curves of quick-frozen rat liver reveal the start of thawing at a point corresponding to 0.3 *M* saline, and complete thawing well below the freezing point of plasma (5). Hypertonicity of liver cells relative to blood plasma thus is well-established for the rat, and adequately documented for cat, rabbit, mouse, and guinea pig (6). A relation of this fact to the secretory character of the hepatic cell is suggested by Robinson's discussion of the kidney but remains to be documented. Cell electrolyte composition cannot account for the osmotic pressure of the hepatic cell; it has been suggested that organic compounds in cytoplasm account for most of the osmotic pres-

[1] The following abbreviations have been used in this chapter: BSP (sulfobromophthalein); PVP (polyvinylpyrrolidone); RE (reticuloendothelial).

[2] The opinions or assertions contained herein are those of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Establishment at large.

[3] Over 1000 communications dealing with this subject were seen, even after ruling out communications of predominently medical or biochemical import. (Period covered May, 1952 to May, 1955.)

sure difference between liver cells and blood plasma (2, 5). Osmotic relations involving cytoplasmic inclusions and nuclei cannot be reviewed here.

Liver injury lowers osmotic pressure of rat liver slices (6). Livers of rat fetuses are hypotonic to maternal plasma; adult osmolarity is not attained until body weight reaches 120 gm. (7).

Swelling of liver slices in low potassium media is reduced by addition of plasma proteins, polyvinylpyrrolidone (PVP), or dextran, and is minimal at colloid osmotic pressures of 70 to 85 mm. $H_2O$ or more (8). Similarly, hemoglobin minimized edema and potassium loss in perfused rat livers (9). Bile and blood flow in the isolated rat liver can be maintained by plasma protein concentrations greater than 0.5 per cent (10).

*Extracellular compartment.*—For the adult rat, 18.1 per cent chloride space (11) and 19 per cent sodium space (12) were found in the liver. Fasting resulted in 20 per cent, regeneration (24 hr.) in 11 per cent chloride spaces (11). Inulin space is 20 per cent in guinea pig liver, 28 per cent in slices of the same tissue (2). Sodium rapidly equilibrates with about 20 per cent of rat liver slice volume but continues to permeate the tissue slowly thereafter (13). In perfused rat liver, sodium equilibrates with 19 per cent of the tissue volume in 5 min. and does not permeate further during several hours (12). The biliary tree occupies no more than 1 per cent of the rat liver (14).

*Liver blood volume.*—Simultaneous estimates of erythrocyte ($P^{32}O_4$) and plasma (T1824, iodinated human serum albumin) volumes in the liver gave blood volumes of from 17.8 to 21.7 per cent and tissue hematocrits 0.69 times as great as those in large blood vessels (15, 16; see 16A for effect of anesthetics).

Liver plasma protein distribution spaces as here determined may not measure the volume of plasma in the hepatic circulation. Combining histochemical and immunologic procedures, the distribution of various plasma proteins within the liver was shown, invariably, to include extravascular areas, especially the structures within the Glisson sheath. Some specificity of intracellular distribution was recorded (17). Liver lymph (collected, slightly contaminated with intestinal lymph, in unanesthetized dogs) reflects changes in plasma protein composition and intravenously-administered protein bound dyes within less than 5 min., and is in protein equilibrium with blood plasma by the end of 15 to 20 min. (18).

Since, therefore, various plasma proteins enter very rapidly at least those extravascular spaces in the liver that are drained by lymphatics, it is not surprising to find that the albumin space in the liver approaches the total extracellular space, even if corrections are applied for the constant penetration of albumin into cells. In the rat liver (sodium pentobarbital anesthesia, or perfused), for instance, albumin space is about 15 per cent, extracellular space ($Na^{24}$) 19 per cent (12, 15, 16).

Distribution space for actively circulating blood, estimated from single passage dilution curves for labeled plasma or red cells in the isolated rat liver, shows a red cell space identical with that determined by conventional

means, but a plasma space in accord with the large vessel hemotocrits rather than the low hemotocrit previously reported (12). In all then, it would appear as though in the liver the distribution space for the plasma proteins is substantially identical with the extracellular space, and that the parenchymal cells are bathed by a medium in relatively rapid and complete equilibrium with blood plasma passing through the liver. The red cells by contrast are confined to a space occupying about 10 per cent of the parenchyma.

The anatomical localization of the extracellular phase and of the areas of lymph formation is vague at present. Electronmicrographs have conclusively shown that in the normal liver a space between endothelium and parenchymal cells (Disse space) does not exist (19, 20). The juxtasinusoidal surface of the hepatic cells, as well as that toward the bile canaliculi, displays small protuberances (microvilli) of obscure significance (20). It may be significant that lymphatics accompany the central veins only in species with well-developed hepatic vein sphincters (18, 21, 22).

## Hepatic Circulation

*Functional anatomy.*—The liver lobule, as a basis for circulatory consideration, has lost much of its former usefulness as the result of realization of the platelike arrangement of liver cells, and of careful injection studies demonstrating the confluent, continuous character of the entire sinusoidal bed (23, 24, 25). These observations reveal the liver circulation as a three dimensional spongework of interconnected sinusoids, supplied by arborizing distributing branches of the portal vein, draining into the hepatic veins at all levels [rat (23)] or at the central vein level only [man (25)]. Afferent and efferent vascular trees interdigitate in a dense, three-dimensional pattern (25A). In the rat, the immediate periportal region is poorly and indirectly supplied with blood (23). The hepatic arterial blood reaches the sinusoids partly after passing directly through the peribiliary plexus, partly by way of the portal vein, and partly via arterioles penetrating the sinusoidal bed to empty into the sinusoids at all levels (25). The random vascular pattern of the fetal liver [see (26) for description of development of plates and sequence leading to plentiful vascularization] is converted into the more orderly arrangement of the adult organ by the onset of respiration, possibly under the influence of the pumping action of the diaphragm [(27), where relation of portahepatic venous pressure gradients to lobular appearance is also discussed].

Microscopic observation of the living tissue seems an important tool to connect these anatomic data with the circulatory measurements of the physiologist. Oxygen insufflation was found to be a useful aid to eliminate respiratory movements interfering with accurate observation (28). Measurement of blood flow rates in transilluminated vessels should become feasible by application of recently developed methods (29).

In the normal liver, shunts from portal to hepatic vein have not been observed by any group. Hepatic artery to hepatic vein shunts have been re-

ported by Andrews *et al.* (30) and denied by Friedman *et al.* (31). Approximate vessel dimensions have been estimated in the rat (29, 31): portal venule 25 (30 to 40) $\mu$, hepatic arteriole 15 $\mu$, sinusoids 10 (6 to 10) $\mu$, central venule 40 (30 to 80) $\mu$, sublobular vein 65 $\mu$. By using an indirect method, supravital estimates of limiting vessel dimension for various routes through the liver were obtained and compared to other vascular beds (32). The data suggest the existence of hepatic artery to portal vein shunts, and indicate the absence of the large vascular bypasses seen, e.g., in muscle.

*Pressure relations.*—Portal vein pressures in resting individuals range from 14.8 cm. of saline in the rat [pentobarbital (31)] to from 17 to 19 cm. of saline in recumbent men [anesthetized (33)] as well as conscious (34)]; in each case pressure was referred to the hilum of the liver. Hepatic vein pressures are 10 to 12 cm. saline below portal (34, 35, 53). Hepatic vein pressures recorded when the vessel is occluded by the catheter correlate closely with portal venous pressures, and are about 2 cm. saline lower in normal individuals than in the presence of liver disease (34). Mean sinusoid blood pressure thus seems to depend on portal pressure in the human liver in the absence of cirrhosis. Distribution of the portahepatic venous pressure gradient along the path of blood flow or with reference to hepatic architecture is unknown.

In active patients portal pressures may undergo extreme changes, rising to as much as 100 cm. saline when procedures likely to raise intra-abdominal pressures are undertaken (33). The author comments that in the light of such measurements the concept of portal hypertension clearly requires redefinition (reference to intra-abdominal pressure near hilum?). Using air distension of the intestine as a means of raising intra-abdominal pressures in rabbits, elevation of portal pressure and, presumably, of hepatic resistance to blood flow was produced (36). Screening the liver so as to exclude the direct pressure on the organ eliminated this effect; reflex changes induced by the ileus thus do not account for the resistance change. In man, increasing abdominal tension by pressure bandages results in decreased hepatic blood flow, attributed again to an increase in hepatic resistance (37).

Hepatic resistance has not actually been measured in any of these cases since either total hepatic blood flow or the relation between portal and hepatic arterial flow and portal pressure remained unknown. Yet under conditions such as here described, hepatic arterial flow is most likely to vary. In the rat alone experimental conditions are favorable to eliminating this difficulty without serious liver injury (38 to 41).

*Hepatic blood flow.*—Methods based upon Fick's principle continue to receive attention since they allow measurements in intact subjects. Critical studies of sulfobromophthalein-based techniques include reports on the effects of enterohepatic circulation (42) and of extrahepatic uptake (43, 44, 45). Hepatic blood-flow estimates based upon the original technique will yield results from 10 to 20 per cent too high in normal individuals. BSP distribution studies suggest that in the presence of liver injury hepatic blood

flow may be overestimated to a much more serious and unpredictable extent (up to 60 per cent) as a result of increasing prominence of extrahepatic dye uptake (45).

As representative of radioactive colloids, gold and chromic phosphate colloids have been applied to liver blood flow determinations (46, 47). Their advantages include high extraction efficiency (80 to 95 per cent), eliminating need for catheterization except under the most exacting conditions, rapidity and irreversibility of disappearance from the blood stream facilitating repeated measurements and, in the case of the former, possibility of external measurements requiring no blood samples (46). In man, at least, the uptake is not so rapid that mixing time problems vitiate the blood flow determination (47). A potential disadvantage is the fixation of relatively highly radioactive point sources for long times in the liver. While conclusive evidence on this point is not yet available, especially in relation to the problem in man, available data suggest that the diagnostic doses employed do not pose a serious hazard (48, 49). Extrahepatic deposition here again may lead to minor overestimation of hepatic blood flow in the normal patient. In the presence of liver injury errors may be introduced by abrupt changes in the extraction efficiency (50), and by the fact that the spleen is an important extraction site which may falsify the measurements when hepatic blood flow differs significantly from splanchnic blood flow.

While in general hepatic blood flow, as here measured, does not differ from splanchnic blood flow, other parameters do not admit of such simplification. Thus Bradley, in an ingenious combination of dilution curve techniques with BSP liver blood flow estimates, has determined splanchnic blood volumes (51). Together with the direct hepatic blood volume determinations reported above, these data indicate that almost half of the splanchnic pool is outside the hepatic vascular bed. Similarly, portal vein punctures and simultaneous hepatic vein sampling show that, even in fasted resting men, approximately 40 per cent of splanchnic oxygen uptake occurs outside the liver (52).

Liver perfusion values in resting subjects range around 31 per cent of cardiac output (53), 1400 to 1700 cc./min. (37), or 28.2 per cent of the circulating blood volume (47), tissue perfusion rates being seemingly much alike in man, rat, and dog, namely 1.0 to 1.5 cc./min./gm. liver (but much higher in the chicken).

Hepatic blood flow rate is remarkably labile: posture, drugs (37), food intake, and food quality (54) readily affect it. Reflex as well as intrahepatic mechanisms participate in its control (37). Anesthesia and alcohol effects have been claimed by some (55), and minimized by others (56, 57, 145).

*Vasomotor control.*—Analysis of hepatic vasomotor control calls for a method of recording rapid changes in liver perfusion without excessive trauma to the subject. "Internal calorimetry," based upon the temperatures attained by thermocouples heated by controlled current, and implanted into the substance of the liver, furnishes qualitative information of the required type (58) for the vicinity of the sensing element [see also (59, 60) for discus-

sion of limitations]. This technique has revealed vasomotor reflexes in the liver set off by changes in blood pressure stimulating carotid body and aortic arch pressoreceptors, and mediated via celiac plexus and right vagus, respectively (40, 61). The responses are rather transient, however. Even the denervated liver retains its ability to compensate for changes in portal pressure (62); in general, in the resting liver, blood flow appears relatively independent of innervation (62). The pressoreflexes, however, are likely to be of importance in responses to pharmacologic stimuli such as those discussed by Olerud (37). Intrinsic hepatic vasomotor control is called upon by both groups to account for postulated more protracted changes in liver resistance.

A humoral factor in normal hepatic vasomotor control is suggested by observations in the isolated rat liver (63). The identity of the hepatic vasoconstrictor factor found in fresh rat blood and rapidly inactivated by the liver is not established, although it has been differentiated from a number of naturally-occurring pressor agents. Analogous observations have not been made in earlier dog and cat liver perfusions.

Dog liver perfusions furnish evidence of ganglionic elements within the liver, possibly related to the function of the venous sphincters in this species (64). Similar observations have been made, however, in the rat. Study of the bradycardia of circulatory distress in the fetal lamb suggests a pressoreceptor area in the liver of this species distal to the ductus venosus (65).

*Blood distribution.*—Radioangiography has contributed evidence that blood distribution within the liver, as well as total flow, is highly labile (66). Increased hepatic resistance under elevated intra-abdominal pressures (36), the direct effect of norepinephrine and of celiac stimulation (66) on the liver, but not acute liver injury (67), are among the conditions which result in direct or indirect evidence of "restricted portal circulation." Intralienal administration of radiopaque material has been discussed as a method of producing portal angiograms without excessive surgical trauma (68).

Transillumination studies reveal instability of the hepatic circulation also at a microscopic level. Intermittency of sinusoidal flow has been seen in the rat even after respiratory movement was arrested by oxygen insufflation (28). Sphincters at "both ends" of sinusoids are claimed by Irwin & MacDonald (28), while others report evidence of sphincter action near the outflow end only (31). In the dog, intermittency of sinusoid flow is considered evidence of tissue injury (69).

In the isolated rat liver resistance to blood flow increases (63), and estimated vascular cross section decreases (70), with decreasing perfusion pressure. Analysis of these data suggests that opening pressures (cf. 71) for hepatic blood channels show a distribution around normal portal pressures, such that *in vivo* the hepatic vasculature is in critical balance: minor shifts in blood pressure or in tissue tension result in opening or closing of numerous possible channels.

Future description of circulatory conditions in the liver should include, it would seem, total hepatic blood flow, sinusoid or portal, and hepatic vein

pressure, and some estimate of effective cross section of the vascular bed. A single injection of suitable radiocolloid, and a single hepatic vein catheterization (free, later occlusive), on the basis of the reports reviewed here, can furnish all requisite information.

*Oxygen consumption, ischemia, anoxia.*—While splanchnic oxygen consumption has been measured in various situations (52, 53, 72), hepatic oxygen uptake *in vivo* has not been reported. Liver slices, according to some workers, have an endogenous respiration of about 1 cc./gm. wet wt./hr. (73, 74) [see (74) for use of fiber glass supported tissue slices to eliminate medium effects]. Others report higher values for liver slice $Q_{O_2}$ (3.2 for mouse, 2.0 for rat and dog) under carefully controlled conditions (75). Isolated rat liver preparations (9) have been reported to consume about 2.9 cc. $O_2$/gm. wet wt./hr., a value considerably higher than earlier determinations (as well as recent ones in the reviewer's laboratory), which led to figures near 2 cc./gm./hr. Correlation of liver $Q_{O_2}$ with surface area in different species, suggested by earlier tissue slice data from the laboratories of Kleiber, Field, and Krebs, has not been confirmed by the scattered perfusion data available in dog, cat, and rat, and has been specifically denied by Martin & Fuhrman (75). Attention is drawn in that communication to species differences in relative organ weights as primarily responsible for observed differences in total metabolic rate.

Critical study of glucose release by the liver indicates 12 vol. per cent oxygen content in hepatic vein blood as the limit below which tissue injury occurs in the dog (76). In man, severe anemia is compatible with normal liver function tests (77); blood flow or hepatic vein oxygen content are not reported. Evidence for a relation of the centrilobular lesions of $CCl_4$ intoxication to circulatory factors resulting in centrilobular hypoxia has been reviewed (78); angiographic observations (67), as well as the lack of oxygen saturation information, tend to render the simple theory propounded somewhat tenuous, and suggest that it may represent an example of a type of circular reasoning often encountered in this field.

Devascularization of the liver in stages can be survived by dogs provided dearterialization precedes occlusion of the portal vein (79, 80, 81). While Popper and co-workers feel that, under these conditions, adaptation of the hepatic tissue to anoxic conditions takes place (see other data on this below), others emphasize the rapidity of regeneration of hepatic vascular supply, especially on the arterial side; hepatic blood flow at the end of a series of operations eliminating virtually the entire original blood supply was found to be 18 to 48 per cent of preoperative values (80). No oxygen tension data are given, but experience with the vascularization of neoplasms of the liver (82) would confirm Rappaport's view that the regenerated supply is virtually entirely arterial.

In contrast to the dog, the rat will survive single stage liver dearterialization in nearly all cases even without antibiotics (38). Survival of dogs following acute ischemia is greatly improved by induction of hypothermia in the

liver before and during the ischemic period (83, 84). The effect of reintroducing a liver into the general circulation after an ischemic period is described in (85).

## Reticuloendothelial Elements in the Liver

*"Stationary" phagocytic cells.*—Kupffer cells *in vivo* do not protrude into the sinusoids, but are flush with the endothelial lining (28) [but see (20)]. A constant turnover of these "stationary" cells is indicated by the slow but constant movement of Thorotrast (25 per cent colloidal $ThO_2$) through the liver: this colloid moves, mostly within macrophages after the first day, from periphery to liver (where it stays longest) to lungs, whence some of it is excreted into the respiratory tract (86). Within the liver $ThO_2$ undergoes cycles of uptake by Kupffer cells, release upon their disintegration, and renewed uptake (87). These data may contrast with results on $CrPO_4$ colloid which, once taken up, remains in liver and spleen as long as it can be followed (88).

The lability of the Kupffer cell population in the liver is exemplified by the extrusion of recently absorbed $CrPO_4$ colloid following thermal injury in dog (50). The extruded material, on reinjection into other dogs, is cleared but slowly; radioangiographs suggest its association with polymorphonuclear leucocytes. A humoral factor seems responsible for this extrusion phenomenon. In the developing rat Thorotrast uptake studies reveal the gradual emergence of fixed phagocytic cells in the liver, reaching adult levels only in the 40-day-old animal (89).

There is need, in attempts to describe the functional status of the RE system, of a means of estimating the active Kupffer cell population at a given time.

*Uptake of radiocolloids.*—Radioactive colloids of suitable micelle size are removed from the circulation largely by liver and spleen.

Colloidal gold (90), $CrPO_4$ (91), polyphosphate (92), Thorotrast (86), and possibly iron sols (93) fall into this category, while the sols formed on injection of certain rare earth solutions into the blood stream have micelles too small for efficient hepatic uptake (94, 95).

Disappearance curves after intravenous $CrPO_4$ colloid injection display a dominant, fast exponential component representing uptake by liver and spleen, and slower components resulting from extrahepatic uptake in various regions (91). A nonextractable component, also suggested by these data, was found to account for 1 to 3 per cent of the radioactivity of this preparation when studied in isolated rat liver preparations (70). The constant extraction efficiency for the remaining, polydisperse 97 to 99 per cent is explained by studies with gold colloids. These frequently fail to show the simple exponential blood disappearance curves of Dobson's chromic phosphate suspensions which are attributable to the presence of micelles smaller than 30 $m\mu$; for these, extraction efficiency decreases rapidly with size, while larger particles are taken up at a rate nearly independent of size (90). Distribution of injected radiocolloids likewise depends on micelle size, larger

components lodging chiefly in liver and spleen, while bone marrow becomes important in the uptake of small micellar colloids (93, 94, 95).

Saturation of the uptake capacity of the hepatic RE system is not approached if diagnostic doses of radiocolloids are employed. Repeated injections yield identical clearance curves (46, 47, 92). India ink administration fails to alter gold colloid disappearance curves in dogs (96). On the other hand, relatively large doses of Au (46) or $CrPO_4$ (93) colloids have been reported to impair subsequent blood clearance of test doses of the same preparations. Thorotrast distribution and excretion in mice is not changed by repeated doses until enormous amounts (1.4 gm. $TO_2$/15 gm. mouse!) have been accumulated (97).

If thus the question of saturation of colloid uptake by Kupffer cells remains problematic, significance of "RE blockage" as a tool for demonstrating participation of these cells in any particular reaction is even more uncertain. In at least one case (India ink) it has been shown that the effects of the "blockage" can be reproduced completely by the particulate free suspension medium (96). For several of these blocking agents direct toxic effects upon the hepatic parenchyma have been reported [India ink (96); indigo carmine (98); trypan blue (older data)]. Deductions based upon functional changes following administration of so-called RE blocking agents appear inadmissible for the present; if such data are combined with more direct demonstration of Kupffer cell participation (e.g. 98, 99) they should prove valuable to future resolution of this problem.

Circulatory conditions, in the isolated rat liver, affect colloid extraction efficiency measured by $P^{32}$-labeled $CrPO_4$ (70). The relations can apparently be analyzed in terms of first order reaction kinetics in such fashion that the extent of $CrPO_4$ extraction is a measure of mean transit time of blood through the liver. Coupled with known blood flow rate, measurement of $CrPO_4$ extraction efficiency thus allows estimation of relative cross section of the hepatic vascular bed.

The relation between linear blood flow rate and amount of $CrPO_4$ colloid extracted may account for the fact that *in vivo*, in the rat, $CrPO_4$ is deposited largely in the periphery of the lobules (100), while in the perfused rat liver, and also in the mouse liver (101), a more uniform distribution is seen. Polyphosphate shows the same distribution pattern in the rat as $CrPO_4$ (102).

Alteration of the surface properties of gold colloids, e.g. by coating with gelatin, or more markedly with gelatin and human plasma fraction IV-4, retards their disappearance from the blood (103, 104).

Polyphosphate extraction is delayed by administration of polyvinylpyrrolidone, dextran, or heterologous plasma. The timing of the effects suggests that the presence of these plasma expanders in the circulation, rather than any previous uptake by RE cells, accounts for the observed effects. The authors, however, prefer to interpret their data in terms of RE blockage. PVP was shown to reverse, in part, already accomplished polyphosphate uptake (105). Whether this is evidence of PVP shock in dogs, and hence

analogous to the thermal injury extrusion phenomenon, cannot be determined. In the absence of data on liver blood flow, interesting clearance data for colloids (such as 106, 107, 108) cannot be interpreted in terms of RE activity, or extraction efficiency.

Before leaving the radiocolloids, attention might be called to their use as sources of internal irradiation (48, 49, 109), and to the observation that plasmapheresis appears to sensitize dogs to the noxious effects of such exposure (109), leading to cirrhosis and ascites. The total dose of radiation thus accumulated before injury becomes manifest is very high, indicating rapid tissue recovery during such exposure.

*Kupffer cells in lipid uptake.*—The role of Kupffer cells in uptake of blood lipids is being explored actively. Ingestion of cholesterol, but not injection of cholesteremic serum, results in rapid accumulation of this substance in the liver (110). Birefringent material, said to be cholesterol, simultaneously appears in the Kupffer cells. RE "blockade" prevents this accumulation in the liver as well as appearance of birefringent material in the Kupffer cells (99, 110). Isolation of Kupffer cells, by a combination of the ingenious trick of Rous, and the technique of Anderson (111), confirms by analysis accumulation of cholesterol in these cells (112).

Dyed fat emulsions, on the other hand, disappear from the blood stream, and become demonstrable in the parenchymal as well as in the Kupffer cells (98). RE "blockade" in this case is said to prevent uptake by the Kupffer cells, but not by the parenchymal cells, nor to affect the plasma disappearance curve of the emulsion. The role of the emulsifying agent employed in preparing emulsions, and the relation of observations like those detailed above to the fate of chylomicrons, is under active investigation in several laboratories.

## Bile Secretion

*Structural aspects.*—Bile canaliculi emerge as separate distinct structures from teased preparations (Elias, 1949). Electron microscope studies (19, 20) fail to reveal distinct walls for these intercellular canaliculi; they appear bounded by the walls of the parenchymal cells proper. Chance observations of stretched preparations may clarify this apparent contradiction: hepatic cells partially pulled apart adhere most firmly in the immediate vicinity of the bile canaliculi; the fragments of cell walls bordering this region often remain intact together in a teased preparation, giving a semblance of a nonexistent distinct wall surrounding the bile canaliculi (19). Fluorescein, as well as alkaline phosphatase, distribution further elucidate the relations between bile canaliculi and sinusoids (113). Especially near branching of the hepatic cell plates, extensions of bile canaliculi to the immediate vicinity of the blood stream occur. Even in such cases, however, a thin but apparently impervious membrane separating blood and bile must exist; neither Edlund (113) nor Hanzon (114) has observed interchange of even such diffusible dyes as fluorescein between bile and blood in such regions, in spite of great concentration gradients. The interconvertibility of bile duct and hepatic cells

has been discussed with some emphasis on the role of mucoproteins and alkaline phosphatase as identifying cholangiomatous growths (115).

*Physiological aspects.*—Höber's distinction between substances secreted into bile in high concentrations and those appearing in bile at levels prevailing in plasma has been confirmed in continuous infusion studies in dogs (116). Hydrocholeretic agents reportedly enhance the rate of excretion of the latter, but not of the former class of compounds (117). A theory has been proposed on this basis, that filtration accounts for transfer of the latter, secretion for that of the former group of substances. The suggested sharp distinction between two classes fails to be borne out by the data presented which indicate a continuous chain of "plasma clearances" from inulin with 0.001 cc./min., through compounds with clearances of 0.003, 0.005, 0.01, 0.03, 0.2, to sulfobromophthalein and bilirubin with 1.5 cc./min.

Filtration as a major mechanism of bile formation seems ruled out by data concerning the relation between bile flow, bile secretion pressure, and blood pressure delivered to the liver. In the rabbit, bile flow is unaffected by deflection of portal blood from the liver (36). Though no sinusoidal blood pressure or hepatic blood flow data are presented, it seems certain that this procedure greatly reduces both. Increasing intra-abdominal pressures (by inflation of the intestines) sharply decreases portal venous flow but does not reduce bile flow significantly until pressures approach 20 cm. $H_2O$. Shielding the liver against the mechanical effects eliminates even this decrease, and bile flow remains constant until intra-abdominal pressures reach 40 cm. $H_2O$. Portal angiograms indicate that decreased bile flow in these cases is associated with "restricted" intrahepatic circulation in Daniel & Pritchard's sense. In the isolated rat liver, bile secretion pressures always exceed perfusion pressures by a considerable margin—an observation wholly inconsistent with a filtration mechanism. Bile flow, here again, was shown to be independent of blood flow at physiological perfusion pressures (118) and proportional to the fraction of the vascular bed remaining open at low perfusion pressures (119).

While relatively independent of portal venous supply, bile flow is highly dependent upon hepatic arterial supply (39). In the isolated rat liver perfused with erythrocyte-free blood plasma at oxygen tensions ranging from 1 to 4 atm., bile flow became progressively less dependent upon perfusion rate or pressure: at 4 atm. $O_2$ bile flow remained constant at all perfusion rates above 0.5 cc./gm./min. (119). Thus, transfer of water and nonconcentrated electrolytes, as well as that of substances secreted against concentration gradients into bile, represents active transport, dependent upon metabolic energy derived apparently from aerobic metabolism.

This conclusion is supported by the temperature dependence of bile flow. In the isolated rat liver, under conditions allowing maintenance of constant blood flow, a temperature optimum for bile production was found at about 39.5°C.; irreversible impairment occurs above 43°C. Bile flow decreases rapidly with decreasing temperatures below 39°C. and ceases irreversibly near 23°C. (118). The data obtained resemble in form those of Hanzon

(114) on fluorescein excretion rates, estimated from intravital microscopy; here, too, 23°C. seemed to be the cutoff temperature.

Sodium, potassium, and chloride ions pass rapidly from blood to bile (14). Isotopically-labeled plasma potassium ion apparently reaches bile without equilibration with intracellular potassium in the isolated liver, even though exchange between blood and liver potassium proceeds rapidly. Phosphate ion, by contrast, is transferred to bile slowly, in low concentrations, and only after interchanging with liver cell phosphates (14). Clear decision between extracellular or transcellular routes from blood to bile is not possible from these results. The phosphate data seem to preclude extensive exchange between blood and bile distal to the region of primary bile formation. The same conclusion was reached from *in vivo* observations of fluorescein concentrations along intrahepatic biliary tracts (120).

*Choleresis, bile salts.*—Compounds displaying marked species specificity in their choleretic action may be of use in clarifying the mechanisms underlying choleresis. A new compound, dibutol, has been added to this small group of drugs (121); administered parenterally, it is an effective hydrocholeretic in the rat, but ineffective in the rabbit.

Differences between the effects of hydrocholeretic and of choleretic agents can be reproduced in the isolated rat liver perfused through the portal vein only (122). Increased hepatic arterial flow, therefore, cannot be responsible for the difference; indeed, no change in blood flow or in oxygen consumption occurred during bile flow response to these drugs. Limited capacity for bile secretion is suggested by the response of bile flow to increasing doses of choleretics (123, 124). Repeated or large doses of cholate or dehydrocholate in the isolated rat liver, or in rats with seven-day-old bile fistulae, result in loss of responsiveness to further doses of choleretics in spite of normal bile flow rates (124).

A most important advance in this field has been synthesis of $C^{14}$-labeled bile acids (125). Using this tool, it has been shown that parenterally administered cholate is recovered from bile almost wholly in the form of taurocholate (126). Less highly oxidized bile acids appear in bile partly or completely hydroxylated as well as conjugated (127, 128). No experiments have yet been reported with doubly labeled taurocholate to establish whether this compound is broken up and resynthesized in transit through the liver. Theoretical interest attaches to this since, wherever detailed studies have been performed, substances concentrated in bile were found to be subject to "detoxication" or transformation reactions in passage through the liver [see BSP, (45); bilirubin (129); as well as bile acids (126, 127, 128)].

Cholate excretion is generally equated to cholate synthesis in the bile fistula animal. By this token, cholate production in the dog is enhanced by feeding excess of any of a number of essential amino acids in addition to a basal diet (130). Various D-amino acids, as well as a number of other dietary supplements, are ineffective in this regard (131). In the fasting dog, cholate excretion is positively correlated with BSP clearance (132). Transfusion of cholesteremic serum in the dog (obtained from animals treated with propyl-

thiouracil) fails to increase cholate excretion (133). In the rat, cholesteremic serum (obtained from donors with bile duct obstruction) raises bile cholate output (134). In the absence of blood cholate data, these results in the rat are hard to evaluate. Should the authors' conclusion be upheld, an interesting species difference, and possibly evidence of a homeostatic control of cholate synthesis in the dog, would seem to be indicated [see (128, 135, 136) for evidence that bile acids are the main end product of exogenous cholesterol metabolism in the rat].

*Bile-seeking dyes.*—Complex formation of many of these with plasma proteins has long been established. Filter paper electrophoresis allows rapid estimation of the proportion of protein-bound BSP in sera (137). In general, parallelism of this value with plasma albumin concentrations was found in patients with hepatic as well as with extrahepatic disease (138). The author suggests that extrahepatic factors may influence the rate of BSP clearance on the theory that protein-bound BSP is more readily taken up by the liver than is the free form. This concept has been further defended by experiments with indigo carmine and with trypan blue in which protein-staining granules were reported associated with dye concretions in hepatic and in Kupffer cells respectively. Since such granules are not found in livers not exposed to these dyes, simultaneous uptake of protein and dye is deduced (139). The fallacy of a concept of "quantitatively protein bound" dyes has crept into enough thinking along these lines to warrant calling attention to the equilibrium existing between such dyes and the binding protein, studied extensively by Klotz and others, and illustrated by the pictures of Pezold (137). In contrast to the concept of an embatic effect of proteins on BSP uptake by the liver, tissue slice and perfusion data [Brauer *et al.* (1949) and (10)] indicate that, other things being equal, albumin in the extracellular phase reduces the proportion and the rate of BSP taken up by rat liver. Diffusion of free BSP, in the direction of a gradient determined by the proportion of free dye in equilibrium with plasma or with intracellular proteins, is proposed as the basic mechanism of BSP uptake by the liver.

The Kupffer cells have been considered as participants in such dye transfer. Their role in regard to soluble, protein-complexed dyes has been made most unlikely during recent years. Liver lymph studies (18) showed liver cells bathed in dye-containing plasma, eliminating any need for intermediate carrier cells. Fluorescein is concentrated in hepatic but not in Kupffer cells, even if time relations are stretched by local cooling (114). $CrPO_4$ colloid uptake and BSP extraction vary independently in the isolated rat liver (10), as do gold colloid and BSP uptake in dogs (96) and in man (46). The effect of India ink, a favorite RE blocking agent, has been attributed to toxic liver injury, (96) and can be reproduced by particle-free suspension medium.

Limited bile concentrations have been reported for BSP, and for fluorescein (114). There is competitive interference of BSP, bilirubin, and cholate for excretion into bile, BSP ranking lowest on this scale (114). Similarly, choleretics increase excretion rate of water and inert electrolytes, but may

actually decrease PAH, penicillin, or BSP output in continuous infusion experiments (117). The possibility that these effects reflect competition for uptake rather than for excretion was raised some time ago (Mendeloff, 1949, for dehydrocholate/BSP) and finds some support in the time relations of fluorescein disappearance from cells and bile canaliculi if cholate or bilirubin are administered sometime after fluorescein. High concentrations of fluorescein at the juxta-sinusoidal surface of liver cells suggest an active uptake mechanism to Hanzon (114).

In the chick embryo BSP concentration by the liver is demonstrable at least three days before biliary excretion (140). In $CCl_4$-poisoned dogs during continuous infusion of BSP plasma concentrations bear the same relation to liver concentrations as found in normal dogs (45), although biliary excretion of the dye is vanishingly small in the former. Even in the normal dog, and using $S^{35}$-labeled BSP (to eliminate effects of slightly colored derivatives), BSP excretion into bile at all infusion rates is slower than uptake so that increasing amounts of the dye accumulate in stores in the liver (45). Yet, under these conditions bile BSP concentrations and excretion rates are determined solely by the infusion rate and apparently are independent of the amount of stored BSP. Disappearance of BSP and of rose bengal from plasma follows a simple exponential course to 80 to 90 per cent disappearance, slowing thereafter (141, 142, 143). BSP extraction is more efficient at slow blood flow rates (38, 53, 184). In cirrhotic livers (144, 145) BSP extraction is reduced, possibly as a result of reduced vascular cross section and consequent short transit time. Anesthesia affects neither estimated hepatic blood flow nor BSP extraction efficiency greatly in dogs (145, 146). Anoxia reduces bile flow rates, bilirubin, and fluorescein excretion (114); BSP uptake is not impaired if circulation is restored after one hour's ischemia of the liver but is sharply reduced if more than 90 min. elapsed before restoration (147) (see bile stasis for bilirubin studies).

### Functional Mass of Liver: Reference Data

Liver function is dependent upon the mass of functional cellular elements available as well as upon circulatory factors and regulatory mechanisms which define the instantaneous level of performance of individual cells. The question of a reference base for such considerations has been weighed in an illuminating discussion (148) in which tissue weight, dry weight, nitrogen content, and deoxyribonucleic acid content in turn were scrutinized. The distribution of the cell population of the liver among different types, and changes in the process of carcinogenesis are described in (149). The problem of polyploidy of hepatic cell nuclei was again discussed (150). The collagen content of rat livers has been determined as a function of age and during regeneration, and fractionated into what appear to be anatomically meaningful fractions (151).

Definition of a functional mass of the liver is complicated by the fact that definite gradients exist within the different zones of liver lobules. While in the case of colloid uptake (100, 102) and of fluorescein uptake and excretion

(114) this may reflect circulatory factors alone, histochemical data show significantly higher concentrations of succinoxidase, glucose-6-phosphatase, succinic dehydrogenase, esterase, and alkaline phosphatase in the periphery of the lobules (152 to 157).

*Variability of enzyme concentrations in liver.*—This has been studied in relation to starvation and protein depletion (158), specific amino acid deficiencies (159, 160), choline (161) and cholesterol content (162) of diets, and to the course of liver growth during fetal development (163) and liver regeneration (164, 165, 170). There is some suggestion in these studies that enzyme systems associated with intracellular-formed elements are less variable than those in solution in the cytoplasm.

## Miscellaneous

While important contributions have been made concerning the relation of the liver to almost all phases of intermediary metabolism and its role in maintaining homeostasis of body fluids, this work is discussed more profitably elsewhere in its proper biochemical context. The present review will, instead, briefly call attention to a number of relatively disconnected but important fields of study relating to the pathological physiology of the liver, and to its role in integrative physiology.

*Inactivation of hormones.*—Inactivation of hormones by the liver has been demonstrated for insulin (166), thyroxin (167, 168), cortisone (169, 170, 171), glucagon (172), and possibly vasopressin (173). In none of these cases has it been shown that such inactivation is of primary importance in maintaining endocrine balance, and, in the case of pitressin, the contrary opinion has been expressed (173).

*Bile stasis.*—Bile stasis results in steatorrhea because of increased intestinal fat excretion in the absence of bile salts in the lumen (174 to 177). The hypochromic macrocytosis of biliary obstruction is minimized by parabiosis with a normal mate, by pregnancy with a viable fetus in utero, or by a spleen extract (178). The existence of two distinct pigments responsible for "direct" and "indirect" van den Bergh reaction has been demonstrated (179, 180) and the relation of blood levels to the occurrence of the direct form, as well as the sites of such transformation, are being explored (129, 179, 180). Histamine is released by the liver of the dog during bile stasis or exposure to bile salts (181, 182).

*The liver in hemorrhagic shock.*—The liver in hemorrhagic shock receives a reduced blood supply (183). The reduction after moderate hemorrhage is in proportion to the cardiac output (53). The importance of vasoexciter material (VEM), vasodepressor material (VDM), and bacterial growth in the development of irreversible shock is discounted by Wayne *et al.* (184) on the basis of experiments, including supplementary liver perfusion in dogs. Pooling in the splanchnic region and increased portal pressure are likewise unrelated to irreversible shock (185). Interesting transillumination studies of the liver circulation during hemorrhage are reported (31).

*Adaptive changes.*—Exposure of rats to relative hypoxia results in initial

metabolic derangements in the liver (186), possibly related to mountain sickness (cf. 187). Adaptation to such conditions result in metabolism associated with elevated liver slice $Q_{O_2}$ at 1 but not at 0.2 atmospheres of oxygen (188). Possible adaptive changes to anoxia were also considered in connection with the resistance of liver homogenate respiration from hibernators to anoxia (189), and with liver function in severely anemic patients (77). Adaptation to test diets in relation to liver enzyme complement was discussed above (158 to 165). Adaptation to cold is associated with exceptionally marked increases of liver $Q_{O_2}$ (190, 191), as well as with initial glycogen depletion followed by glycogen enrichment and liver hypertrophy (192). The question has been discussed whether these changes are of a secondary character or whether they reflect a primary role of the liver in cold adaptation (192; cf. 193). The hepatomegaly seen in some Eskimos is apparently related to diet alone (hypervitaminosis A) (194).

*Regeneration of liver following partial hepatectomy.*—Regeneration does not depend specifically upon portal venous blood supply, but rather upon the total volume of blood flow reaching the liver (195, 196). Liver plates two cells thick are invariably found in regions of liver regeneration and of mitosis [Elias (197); cf. (198) for distribution]. The earliest changes observed in livers following partial hepatectomy are increased respiratory rate (199) and tissue amino acid concentration (11). Changes in various enzymes (164, 165, 170) have been reported, and the relatively slow increase in the mitochondrial fraction during liver regeneration pointed out. While ribonucleic acid turnover increases early, deoxyribonucleic acid responds only late during regeneration (200). Cold exposure enhances (201) whereas cortisone inhibits liver regeneration (202). Liver function during regeneration was studied in relation to uptake of fat emulsions [increased (203)] and, indirectly, by changes in plasma protein levels [albumin and $\alpha_1$ globulin decreased (204)]. An effect of an embryo heart extract on fatty infiltration of regenerating liver had been reported (205). Collagen distribution and concentration during regeneration are reported by Harkness (206).

*Copper and iron metabolism.*—The distribution of injected iron saccharate between ferritin and hemosiderin, and the relative availability of these iron stores for hemopoiesis has been examined in the rat (207, 208). Several workers report on the importance of dietary composition in permitting excessive iron absorption, and deposition in the liver (209, 210, 211). While these workers in general see no histologic changes around hepatic iron depots, marked lesions have been produced in the liver by repeated large doses of saccharated iron given intravenously (212). Serum iron and iron tolerance determinations have been discussed as diagnostic aids (213, 214, 215).

Hepatolenticular degeneration (Wilson's disease) has been associated with decreased plasma and increased urinary copper content (216). The lesion is associated with decreased ceruloplasmin levels in plasma, and possibly with impaired transfer of recently absorbed, albumin-bound copper to ceruloplasmin (217). The liver may be involved in ceruloplasmin synthesis

as well as in the transfer reaction. A role other than that of a mere copper carrier has been surmised for ceruloplasmin (218).

*Physiology of cirrhosis of the liver.*—Circulatory changes in cirrhosis have been measured by Bradley *et al.* (144) and Sessions *et al.* (145). The development of the restricted circulation has been traced angiographically (219), and the anatomic features underlying the intrahepatic shunts contributing to liver dysfunction under these conditions have been beautifully described by Popper *et al.* (220). The association of the fibrosis with the regions of maximal cell injury (221), and of ground substance with the cellular portions of the fibrous bands (222) appears to be well-established. The reversibility of the early fibrotic lesion has been documented again (220), and observations on collagen resorption in the liver may contribute to an understanding of the mechanisms involved (223, 224).

The suspected connection between hepatic coma and blood ammonium levels has been confirmed by demonstration of elevated blood $NH_3$ levels in cirrhotics approaching coma (225, 226), and of reduced ammonium tolerance in cirrhotic patients (227). Catheterization experiments have revealed reduced portahepatic-venous ammonium gradients as well as reduced hepatic blood flow rates in cirrhotics (228). These results were attributed in part to intrahepatic shunts deflecting blood from the sinusoids and close contact with liver parenchyma.

Cirrhosis of the liver is frequently associated with increased cardiac output (229, 230, 231). Ascites and edema formation under these conditions represent an isotonic expansion of extracellular fluid (232). Several workers find evidence of abnormal water and electrolyte metabolism, even in compensated cirrhosis, and feel there is evidence of increased antidiuretic activity unrelated to blood volume or tonicity (233 to 236). Since to date, earlier evidence of faulty metabolism of antidiuretic hormone has not been duplicated (173, 234), the cause of this fluid retention is obscure. Equilibria between plasma and ascitic fluid proteins have been studied (237, 238). Armstrong has shown that plasma colloid osmotic pressures in cirrhotic patients cannot be calculated from electrophoretic data, but frequently fall far below calculated values, and may change greatly without comparable changes in the electrophoretic or total protein analyses (239, 240). He considers the possibility of qualitative changes in serum proteins elaborated under these conditions [cf. (34, 37, 241) concerning "effective portal pressure"].

Within the space allotted, the present review cannot cover more than a selected number of topics. In closing, attention may be called to a few other areas of importance entirely comparable to those here considered and to more or less extensive discussions of these: (*a*) Toxic liver injury [reviewed by Drill (78)]: important recent trends—association of specific biochemical lesions with a few agents (Sedormid, allyl formate, ethionine); indirect effects via increased nutritional requirements (ethanol, possible x-radiation in relation to cirrhosis); demonstration of possibly hepatotoxic character of frequently used drugs (thiobarbiturates, chlortetracycline); (*b*) hepatomata:

causes and histogenesis [recent conference (242)]; (*c*) nutritional factors in liver injury [especially differentiation of nutritional cirrhosis and liver necrosis. See conference outlining progress (243), and recent review (187)]; (*d*) hepatitis of viral origin [note etiology and development of mouse hepatitis (244, 245, 246), localization of virus during development of canine virus hepatitis (247), and numerous contributions dealing with liver function and with recovery)]; (*e*) Plasma protein production [see especially isolated organ experiments (248, 249)]. Finally, refer to the final Macy Foundation conference on liver injury for discussions of the role of the liver in carbohydrate and in fat metabolism (250).

## Addendum

Since completion of the original manuscript the author's attention has been called to several reports which appear of interest in relation to problems discussed above.

In connection with the physiological role of the hepatic arterial supply, and possibly, with the question of materials transfer from blood to bile, recent results of Andrews, and Maegraith & Richards in the isolated dog liver preparation (251) may prove important. These authors showed that extraction of BSP from perfusate is over twice as efficient in the organ perfused through the hepatic artery as in the same organ perfused at the same rate through the portal vein. Yet, if the liver is perfused via both channels, the higher ("arterial") extraction efficiency is also observed. These authors consider the possibility that the differences in extraction efficiency described may point to an important role of the peribiliary arterial plexus in the excretion of substances like BSP into the biliary tree. Although the present author has some reservations on this interpretation [an attractive alternative being an interpretation in terms of blood flow distribution related to that discussed for $CrPO_4$ clearance data presented by Brauer *et al.* (69)], these results appear of considerable potential interest clinically as well as in relation to vascular distribution patterns in the liver.

Bennhold and collaborators have greatly extended their early observations concerning association of albumin and of lithium carmine uptake by liver. In the mouse, this dye is taken up preferentially by Kupffer cells; association of dye granules with protein staining deposits was reported in mouse liver. Subsequent experiments with labeled plasma proteins showed, after 12 hr., increased uptake of labeled albumin by the RE cells of carmine treated animals, compared to undyed controls (252). The assumption originally voiced, that this reflected the carrier role of the plasma protein, seems negated by more recent data indicating a great discrepancy between the rapid time course of dye uptake, and the much slower one of protein accumulation. These experiments, however, led to further investigation of dye and plasma protein distribution among subcellular fractions in such labeled and dyed livers. The dye was found associated preferentially with the mitochondrial fraction, and mitochondria were shown to strongly adsorb carmine (and other bile seeking dyes) *in vitro* (253). These data may repre-

sent a morphologic definition of the "relatively inert" storage compartment postulated [cf. (45)] for the transfer of BSP through liver cells. Furthermore, by combination of immunologic methods and a special micro-paperelectrophoresis technique, suggestive evidence for the accumulation of parenterally administered $S^{35}$-or $I^{131}$-labeled plasma albumin on rat liver mitochondria could be obtained under conditions eliminating intravascular plasma protein as a source of error (254). Thus the data of Gitlin and collaborators (17) have been confirmed and extended, and intracellular presence of unaltered plasma proteins in the liver cells appears increasingly well established.

Application of an ingenious method for preparing $P^{32}$ labeled microspheres of the specific gravity of blood (255) have led to more quantitative demonstration of streamlining and imperfect mixing of venous blood in the splanchnic bed (256). An excellent review of the uptake of inorganic colloids by reticuloendothelial cells has been presented (257).

Finally, two interesting additions to present understanding of liver function tests have appeared. In rabbits determinations of $L_{max}$ for BSP have shown that, other factors being equal, $L_{max}$ decreases in direct relation to the liver mass if this is modified by partial hepatectomy (258). This interesting finding should not, it is hoped, mislead other workers into applying similar reasoning to estimate "residual hepatic mass" in diseased livers. Other factors, discussed in the appropriate section above, dominate BSP uptake under those conditions.

A first attempt to isolate factors determining the values of quantifiable liver function tests has been made on the basis of extensive clinical material, and has resulted in a proposal that four different major factors, as yet identified only as statistical correlating factors, underlie the results of liver function abnormalities in cirrhotics and in infectious hepatitis cases (259). It is to be hoped that the next few years will see a fusion of clinical and experimental results in this field and lead to at least a tentative analysis of liver function in terms of measurable components.

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# CENTRAL AND SYNAPTIC TRANSMISSION (PHARMACOLOGICAL ASPECTS)[1]

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The Editorial Board decided recently that one chapter in each volume of the *Annual Review of Physiology* should be devoted to the aspects of some topic which had been particularly illuminated by pharmacological analysis. This chapter has been prepared as a result of that decision, and the emphasis throughout is on the contribution made by such pharmacological analysis to physiology; no attempt has been made to deal with the detailed mode of action of any drug or group of drugs.

The recent publication of the first symposium to be held under the title "neuropharmacology" (1) marks, perhaps, the increasing interest in the pharmacology of the CNS (central nervous system). Since the pharmacological aspects of any one facet of physiology will be the subject of a chapter in this book only once in a number of years, the scope of the review has not been restricted to the literature of the current year, and earlier publications have been freely quoted in order to provide a background to more recent developments. The review covers the literature available up to May, 1955.

The definition of a synapse has been restricted to a junction between two neurones, thus excluding the neuromuscular junction from consideration. Whenever possible, an attempt has been made to arrange the subject matter according to individual types of synapse. This is now possible not only for autonomic ganglia, but also for the spinal cord and even, to some extent, for the brain.

The interest of a pharmacologist in synaptic transmission is naturally stimulated by the concept of chemical transmitters, and no apology is offered for treating the whole question on this basis.

## Content of Possible Transmitter Substances in Central Nervous System

*Acetylcholine, cholinesterase, and cholinacetylase.*—The presence of ACh (acetylcholine) in brain and spinal cord has long been recognised, and MacIntosh (183) in 1938 carried out detailed quantitative studies of its localization. Feldberg (94) has interpreted these data and the similar distribution of ChA (cholinacetylase) in terms of an alternation between

[1] The following abbreviations have been used in this chapter: CNS (central nervous system); ACh (acetylcholine); ChA (cholinacetylase); ChE (cholinesterase); DFP (difluorophosphonate); ATP (adenosinetriphosphate); 5-HT (5-hydroxytryptamine); LSD (lysergic acid diethylamide); CSF (cerebral spinal fluid); DNP (dinitrophenol); EEG (electroencephalograph, electroencephalogram).

cholinergic and noncholinergic neurones in the CNS, and he has recently discussed the implications of this hypothesis again (95). The basis of the hypothesis is that the dorsal roots and tracts are low (primary afferent), the lateral filament and spinocerebellar tracts are high (second afferent), the internal capsule is low (third afferent), the motor cortex is intermediate (a number of interneurones), the pyramids are low (upper motoneurones), and the motor roots and nerves are high (lower motoneurones) in ACh and ChA content.

Further confirmation of some of these facts has recently been obtained by Wolfgram (246) who found 10 to 20 μg./gm. of ACh in the ventral roots of cattle, a quantity more than 100 times the average content of dorsal roots from the same animals.

A number of authors (85, 219, 236, 242) have shown that anaesthesia and central depressants cause a rise, and exercise and central excitants a fall in the ACh content of the cortex. The effect of anaesthetics in causing a rise in ACh content has recently been confirmed by Crossland & Merrick (64), who found that the increase was general in all cell areas and that no differences were detectable between the different anaesthetics used. Such changes can only be observed when careful precautions are taken to ensure the immediate inactivation of ChE (cholinesterase) and ChA in the brain. Stewart (230) and Michaelis *et al.* (194) described a twofold rise of ACh in the cortex of rat and rabbit brain respectively after DFP (difluorophosphonate), while Robinson *et al.* (220) obtained a similar rise in both brain and spinal cord which was not prevented by atropine.

MacIntosh & Oborin (184, 185) have recently carried out an elegant series of experiments on the release of ACh from the intact cortex of the cat. They collect, in a small perspex cup containing eserinised saline, the fluid which exudes from the exposed cortex at a rate of about 0.02 ml./cm.$^2$ cortex/min. Under light ether anaesthesia, ACh in the cup reaches a concentration of $3\times10^{-7}$. The amount of ACh is reduced by 50 per cent by increasing the depth of ether anaesthesia, and to zero by chloralose anaesthesia, and is roughly proportional to the electrical activity of the neighbouring cortical areas.

MacIntosh & Oborin (185) have also investigated the source of ACh. The release is not affected by varying the blood flow and is not derived from postsynaptic parasympathetic fibres. No indication was obtained that the ACh was released from afferent fibres reaching the cortex from lower centres; yet the release was abolished by completely undercutting the cortical area. The ACh is probably released by cortical interneurones activated by afferents from lower centres.

The distribution of ChE in the central nervous system can be studied much more accurately than that of ACh by using histochemical localization. This technique owes much to the work of Koelle, who has recently published (155) a complete study of the cat. Koelle's data agree well with the previous work done on the dog by Burgen & Chipman (47). Pseudo ChE (nonspecific; butyryl) is located almost exclusively in the connective tissue in the glio-

cytes and capillary walls; true ChE (specific; acetyl) is differentially present in certain neurones and is located on the walls of cell, axon, and dendrites. Motoneurones, known to be cholinergic, contain large amounts of true ChE, and similar quantities are found in tertiary afferents and in some correlation centres; on the other hand, primary afferents, considered on other grounds to be noncholinergic (94, 95, 183), contain very small amounts of true ChE, as do other correlation centres. Secondary afferents contain intermediate amounts. Moderate amounts of the ChE were found in the supraoptic nucleus, and other evidence has been adduced (71) that ACh acts there as a transmitter for the release of antidiuretic hormone. The pattern which Koelle describes thus agrees well with Feldberg's suggestion (95) of an alternation of cholinergic-noncholinergic neurones. Support for Koelle's work is found in that of Cavanagh *et al.* (58), who claim that all pseudo ChE in the central nervous system is located in glial cells; these workers also state that in peripheral nerve the pseudo ChE is not associated with conduction and is probably a component of the cells of Schwann. Wolfgram (246) showed that the same amounts of true and pseudo ChE were present in both ventral and dorsal spinal roots in cattle; ChA activity was, however, more than 20 times greater in ventral roots. This work could be interpreted as indicating that although ChA activity runs parallel with ACh content (ACh immigrating down the axon to the terminals), ChE activity is specifically located pre- or postsynaptically near the site of release of ACh, and consequently will not always be correlated with the ACh content. A view of this kind would run counter to the theory that ACh is involved in nerve conduction (197).

*Sympathin and amine oxidase.*—The concept of an alternation of cholinergic-noncholinergic neurones demands a second chemical transmitter; and even the most enthusiastic advocate of a monist view of chemical transmission will readily admit that there are still no very obvious candidates for this rôle amongst the active principles which occur in the central nervous system. Nevertheless, by analogy with the peripheral autonomic system, first thoughts naturally turn to sympathin, and a major contribution has been made by Vogt (241) to this subject. She found a high concentration of sympathin (1.0 μg./gm.) in the hypothalamus and in the area postrema, rather less (0.7 μg./gm.) in the mid-brain and round the aqueduct, and still less (0.3 μg./gm.) in the medulla and medial thalamus. Nowhere else was a significant amount detected; all myelinated tracts were negative. Thus, apart from the local concentration in the area postrema, all the sympathin is found in those areas containing the central representation of sympathetic activity. In the cat, about 7 per cent, and in the dog about 14 per cent of the sympathin is epinephrine, the remainder norepinephrine. Vogt (241) suggests that the sympathin is the adrenergic activator of secretion from the anterior pituitary and has shown that the content of sympathin in these areas (but not in the area postrema) is reduced by drugs which stimulate these centres, but not by other central excitatory drugs. She further suggests that the sympathin in the area postrema is located in astrocytes, since

Bülbring *et al.* (46) found large amounts of sympathin in gliomata.

Another attack on the problem of the rôle of sympathin has been made by Koelle & Valk (156) with equally negative results. They have developed a histochemical method of localising monoamine oxidase, and on applying it to the cat they found no correlation whatsoever between the site of the enzyme and adrenergic tissues.

*Other active substances.*—Ever since Dale (66) suggested that the substance responsible for the vasodilatation, produced by antidromic stimulation at the peripheral end of a sensory nerve, might be the same as that released at the central end of such primary afferents, attempts have been made to follow up this attractive possibility of identifying a central sensory transmitter. Antidromic stimulation was said to release histamine [Kwiatkowski (164)] and ACh [Wybauw (252)], but Holton & Perry (141) were unable to confirm these claims when studying the phenomenon in the rabbit's ear. Holton & Perry (142) also found that sympathin was not responsible for the effect, and pointed out that there were good reasons for suggesting that the active principle was of large molecular weight (141).

*Adenosinetriphosphate.*—Hellauer & Umrath (128) claimed that from dorsal but not from ventral roots they could isolate an active vasodilator, the enzymic destruction of which was inhibited by strychnine, and which they considered might be the transmitter at synapses of which primary afferents were the presynaptic fibres. Holton & Holton (138) on the other hand, found an active vasodilator in both ventral and dorsal roots. They showed (139, 140) that the substance responsible for antidromic vasodilatation is similar to ATP (adenosinetriphosphate) and have suggested that ATP may be the central excitatory transmitter. Although ATP was present in both dorsal and ventral roots, it persisted only in the former, being rapidly destroyed in the latter. Holton & Holton suggest that a capacity to destroy an active substance is a more important criterion than mere content of the active substance in determining the nature of a chemical transmitter. Yet it has usually been the case that substances which are chemical transmitters are rapidly destroyed, so that one would have expected, a priori, that the destruction would have been more rapid in the dorsal than in the ventral roots.

ATP is found throughout the central nervous system, and Acs *et al.* (3, 4) have studied its metabolism in brain slices. Glucose is the only adequate substrate for its synthesis, which is depressed by glutamate. It is interesting to note, in considering ATP as a possible transmitter, that Falk & Gerard (91) found that ATP applied outside a muscle fibre caused excitation, whereas when it was applied inside the fibre it was inactive. This would appear to suggest some function of ATP in muscle other than a simple source of energy; and the analogy of the experiments to those of del Castillo & Katz (57) with ACh is too striking to be ignored. Moreover, earlier workers, and, more recently, both Babskii & Malkiman (14) and Feldberg & Sherwood (100) have shown that ATP exerts both a stimulant and a depressant action

on the central nervous system. We cannot yet exclude ATP as a possible transmitter.

*Substance P.*—Interest in the work of Hellauer & Umrath (128) has recently been revived by the demonstration by Florey (104) of substances in mammalian central nervous sytem, which have intriguing actions on single stretch receptors of crayfish. An excitatory substance is found in spinal roots and in the cortex which increases the frequency of discharge of the stretch receptor. It acts only when the pH is higher than 7.0 and is enzymically destroyed at pH 7.8. An inhibitory substance is found in the cord (not in the roots) and in the cortex which stops the discharge of the stretch receptor and blocks the discharge excited by ACh. Florey finds similarities between this inhibitory substance and substance P (103). Moreover, Hellauer (127) has now stated that he believes the active vasodilator in dorsal roots to be substance P and not ATP. It is, of course, perfectly possible that both ATP and substance P occur in some quantity in the dorsal roots. The mere presence of either does not prove it to be a central transmitter.

Substance P is present u. (units) (more than 25 u./gm.) in the cortex, area postrema, caudate nucleus, medulla, nuclei gracilis and cuneatus, hypothalamus, mid-brain, and grey matter of the spinal cord [Amin *et al.* (7)]; in large amounts (500 u./gm.) in the ala cinerea [Zetler & Schlosser (254)]; and in small quantities (2 to 8 u./gm.) throughout the mesencephalon and diencephalon [Hausler (126)]. Moreover, Lembeck (168) found substance P in the dorsal but not in the ventral roots of the ox, and Pernow (205) found twice as much in the dorsal as in the ventral roots of the dog. Amin *et al.* (7) confirm these findings, stating that the dorsal roots contain more than the ventral roots, that the dorsal tracts are especially rich, and that enormous quantities occur in the nuclei gracilis and cuneatus. These authors consider that the claims of substance P for consideration as a central transmitter must now be taken seriously. On the other hand, they draw attention to the fact that substance P has no known central action. Yet von Euler & Pernow (89) have found that it does in fact cause a stimulation of respiration on intraventricular injection. Umrath (240) claims that the substance P found in the CNS is not the same as that found in the intestine.

*5-Hydroxytryptamine.*—Amin *et al.* (7) studied the distribution of 5-HT (5-hydroxytryptamine; serotonin) in the central nervous system. They found concentrations of more than $5 \times 10^{-8}$ in the hypothalamus, midbrain, cortical thalamus, and in the grey matter of the cord. It appears to the reviewer, however, that this pattern is so strikingly similar to that found by Vogt (241) for sympathin, that the occurrence of 5-HT is more likely to be related to the central representation of the autonomic system than to central transmission in general. Yet, Twarog (239) has suggested, from evidence on mytilus muscle, that it may have a specific inhibitory action to ACh in this species at least, while Slater *et al.* (228) have suggested it has a rôle as a neurohormone from studies of interneuronal block. Woolley & Shaw (248, 249) have suggested that 5-HT has an important function in mental processes

since drugs such as LSD (lysergic acid diethylamide) which antagonize it produce mental disturbances in men and animals. Lewis & McIlwain (171) found that 5-HT had little effect on the respiration of isolated cortex slices and failed to reverse the inhibitory effect of LSD on this preparation. Poloni & Maffezzoni (213) found that LSD caused an increase in the ACh content of the brain; and Thompson *et al.* (234, 235) that it was a fairly powerful inhibitor of pseudo ChE of human brain. Gaddum (112) and Gaddum & Hameed (113) have studied the antagonism between LSD and 5-HT, and Erspamer (87) has reviewed the rôle of 5-HT and LSD in the CNS.

*Histamine.*—Histamine is found in some nerves, principally in post-synaptic sympathetic fibres, and von Euler (88) has reviewed its location. In the central nervous system, it is not found in high concentration except in the cerebellum (Harris *et al.*, 123) and in the hypothalamus (Kwiatkowski, 164). Kwiatkowski found none in the dorsal roots; Werle & Palm (243) only a little in both ventral and dorsal roots. Hausler (126) has shown in the perfused frog spinal cord, that 0.1 μg. histamine excites the motoneurones; 10 μg. blocks the contralateral reflex. Hausler also claims that after electrical excitation an "H-like substance" is detectable in the perfusate. There is also some recent evidence for a stimulant action of histamine in sympathetic ganglia (159, 237, 238); but, in the opinion of the reviewer, there is little evidence at either site for the existence of histaminergic synapses.

*Pharmacology of the hypothalamus.*—The complexity of the hypothalamus is well illustrated by all these data. As Vogt (241) has pointed out, it contains the highest concentrations in the central nervous system of sympathin, 5-HT, substance P, and histamine; it also contains ACh, ATP, and vasopressin. The possibilities of complex interactions in this pharmacological museum are surely very great and render the multiplicity of the effects of hypothalamic lesions easily comprehensible. These pharmacological studies also serve to indicate the care necessary in interpreting patterns of behaviour which may result from ablation experiments or from the wholesale application of drugs to the central nervous system.

## Transmission in the Spinal Cord

*Sensory afferent–motoneurone synapses.*—Many workers have chosen to study monosynaptic reflexes in the belief that they were selecting the simplest possible transmission system available in the spinal cord. Recent work, however, suggests that the simplicity of the system is more apparent than real, since both components of the reflex are themselves subject to a complicated pattern of influences.

The nature of the reactions of motoneurones was greatly elucidated by the demonstration by Brock *et al.* (35, 36), using intracellular recording, that stimulation of primary afferents from an agonist muscle produced depolarization, while stimulation of primary afferents from an antagonist muscle produced hyperpolarization of the cell membrane. The authors considered that this could only be explained by postulating the release of both an excitatory and an inhibitory chemical transmitter at synaptic knobs on the

motoneurone. Brock & McIntyre (37) have re-examined this suggestion. They were unable to produce any active response to a hyperpolarizing current applied through a double micropipette, while an active response was readily obtained with a depolarizing current. Since, however, inhibition occurred in the former case, even in the absence of apparent current flow, these authors agree that a chemical transmitter may well be involved in inhibition.

Skoglund (227) had earlier reviewed the different effects which anticholinesterases exert on flexor and extensor spinal reflexes, both of which he assumed to be monosynaptic. It was very difficult at that time to envisage how primary afferents were able to exert both inhibitory and excitatory effects directly on motoneurones; and the work of Eccles *et al.* (79), if confirmed, simplifies the concept. According to these workers, the inhibitory effect of primary afferents on motoneurones is exerted only through short interneurones located in the intermediate nucleus; they consider that this interneuronal relay on the inhibitory pathway serves no function other than to alter the type of neurone impinging on the motoneurone. The inference is that all primary afferents release the same excitatory transmitter, but that an inhibitory transmitter is released by the intercalated neurones in the intermediate nucleus. McIntyre (188) does not agree that the direct inhibitory pathway between antagonistic muscles can be disynaptic, in view of the short latency before inhibition is exhibited. Eccles *et al.* (79) argue, however, that latency must be measured to the time of generation of the motoneurone discharge, which Renshaw (218) estimated at about 1 msec. They claim that this is a long enough latency to cover a synaptic delay, and suggest that the safety factor of the second synapse is so high and the lag time so short that the disynaptic reflex has many of the characteristics of a monosynaptic pathway. Eccles *et al.* (80) have shown, using a postsynaptic slow potential as the index of the site of synapses, that Group Ia proprioceptive afferents form synaptic connections only with motoneurones of synergistic muscles and with interneurones in the intermediate nucleus; while Group Ib proprioceptive afferents synapse only with these interneurones and not with motoneurones at all. It is perhaps worth noting that in these experiments, as in all others by Eccles and his colleagues, pentobarbital sodium (Nembutal) was the only anaesthetic employed.

An analysis of the heteronymous connections of afferents was made by Lloyd *et al.* (173), who have suggested that the synaptic connections made by afferents may be of graded "transmitter potentiality" within a group of synergistic motoneurones. Heteronymous reflexes were more easily elicited after cooling. Brooks *et al.* (38) have demonstrated enormous potentiation and repetitive firing, induced by cooling, in monosynaptic reflexes. Howland *et al.* (144) have shown that stimulation of one dorsal root will induce inhibition of the motor response to stimulation of the neighbouring dorsal root. They claim that this inhibition is due to a block of the volley in the afferent fibres before reaching the cells. The alternative explanation of the heteronymous inhibitory reflex, similar to the heteronymous excitatory reflex de-

scribed by Lloyd *et al.* (173) would seem to the reviewer to be more probable.

The pattern of the monosynaptic arc is very much simplified if it can be regarded purely in terms of excitation. The nature of the excitatory chemical transmitter is still, however, completely obscure. All the evidence, and there is now much of it, is against ACh. In the first place, there is the considerable evidence based on the noncholinergic nature of the primary sensory afferents; and in the second place, there is now a good deal of evidence that neither ACh nor the anticholinesterases exert any direct action on the motoneurones. Even in 1948, Eccles (75) was able to collect a formidable body of evidence of this kind. Feldberg *et al.* (97) concluded that ACh, injected into the basilar artery, had no direct effect on the cervical motoneurones, but that it did stimulate "some interneurones." Holmstedt & Skoglund (137) described the striking specific depression of the extensor reflex induced by an anticholinesterase (tabun), given by injection into the aorta close to the origin of the lumbar arteries, and Taverner (233) has more recently studied the actions of physostigmine given by the same route. These effects could well be explained on the basis of a stimulation of cholinoceptive interneurones and a resulting recruitment of inhibitory pathways. Kennard (151) found that ACh, injected by micropipette near the motoneurones, only very occasionally stimulated a reflex response; and his occasional positives could be attributed to a stimulation of some interneurones. Robinson *et al.* (220) found that DFP would block the patellar reflex at the same time as it induced a rise in the ACh and a fall in the ChE content of the cord.

Perhaps the most striking evidence against ACh has, however, been obtained by Eccles *et al.* (78) who showed that although ACh exerted a stimulant effect on Renshaw cells in the ventral horn, it had no such effect on motoneurones as judged by intracellular recording. This work seems to the reviewer to have disposed finally of the possibility that ACh may be an excitatory transmitter at synapses on motoneurones.

The actions of sympathin, which in the past have attracted considerable attention (21, 44, 224), have been shown always to be the opposite to those of ACh (19, 45), but no direct action of sympathin on the motoneurones has been demonstrated. Furthermore, the distribution of sympathin [Vogt (241)] does not support an hypothesis that it has a rôle as a central synaptic transmitter. In the opinion of the reviewer, the actions of sympathin on spinal reflexes are best explained as part of a general modulating activity exerted by sympathin in the cord, in sympathetic ganglia and at the neuromuscular junction. The action of sympathin in the ganglion has been much studied (43, 193, 204), and the importance of the milieu of the postsynaptic cell in determining the action of the transmitter has been pointed out by Loewi (175) and by Perry (207). It may well be that the small amounts of sympathin required to exert such a modulating effect are derived, in the sympathetic ganglion, from short postsynaptic fibres supplying the blood vessels of the ganglion [Lammers *et al.* (165)], and a similar situation may pertain in the spinal cord. A modulating effect of sympathin on transmission in the cord may then be visualized as part of the pattern of autonomic activity in general.

It is also necessary to remember that apart from the factors already mentioned, there are at least two others which modify the monosynaptic reflex, namely, the bombardment of the motoneurone from higher centres and from local feed-back systems. Thus King & Unna (154) have shown that stimulation of the bulbar reticulum can influence the monosynaptic patellar reflex. Bonvallet *et al.* (29) have studied the action of higher centres on the effects of sympathin on the monosynaptic response recorded from a ventral root in response to dorsal root stimulation. After diencephalic section sympathin potentiated the reflex, after retromammillary section it had no effect, and after retropontine section it was inhibitory. These results not only offer some explanation of the varying pattern of response to sympathin observed by other workers (21, 44, 224), they also suggest strongly that the effect of sympathin cannot be a direct one on the motoneurones. Holmgren & Merton (136) have shown that discharge of motoneurones may result in inhibition of other motoneurones probably by a feed-back through Golgi collaterals as suggested by Renshaw (218). Since the Golgi collateral is a branch of a cholinergic axon, it might have been inferred that ACh, released at the terminals of the Golgi collateral, was inhibitory to motoneurones. This is, however, refuted by the work of Eccles *et al.* (78) who have shown that the Golgi collaterals synapse with specialised interneurones in the ventral horn [Renshaw cells (217)], and that this synapse is indeed cholinergic. The inhibitory effect of the discharge of Renshaw cells on the motoneurones is not, however, mediated by ACh. The total latency of this inhibitory response is about 1.2 msec. This work is further evidence that ACh has no direct effect on the motoneurones; that, in agreement with Feldberg's hypothesis (95), one neurone (the motoneurone) liberates only one transmitter (ACh) wherever the axon terminals may be (muscle end-plate or Renshaw cell); and that inhibitory effects on motoneurones are exerted only through interneurones specialised for this function. Eccles (77) has reviewed these and other effects of antidromic stimulation of the motor roots.

Further evidence of the nature of the monosynaptic excitatory transmitter might be obtained from a study of substances which block the reflex. So far no clear-cut evidence has been obtained. Bernhard & Taverner (20) found that tubocurarine blocked the extensor but potentiated the flexor reflex. The inference that such effects are exerted directly on the monosynaptic pathway is not, however, justified; the tubocurarine might have blocked transmission at the specialised interneurones described by Eccles *et al.* (79), or it might have had some action on blocking the feed-back inhibitory system at the cholinergic synapses on Renshaw cells, although Eccles *et al.* (78) found tubocurarine ineffective in blocking at this site. Furthermore, Dun (72) has demonstrated that large doses of tubocurarine, and Frumin *et al.* (109) that procaine can block transmission through the dorsal root ganglion without blocking conduction in the dorsal root peripheral to the ganglion. This cannot, of course, be a synaptic block, and Dun considers the site of block to be the point at which the sensory afferent fibre branches after emerging from the cell body. Gasser (114) has shown that the diameter of the fibre and its conduction velocity change in passing

through the ganglion, and that there is no other change that cannot be explained because of this. There will, of course, be a low safety factor for conduction at the point where the nerve loses its myelin sheath. Strychnine, which now appears to be somewhat more specific than was thought, probably has no direct action on the monosynaptic reflex pathway [Bradley *et al.* (30)].

Malcolm's work (191) on the newborn kitten points the way to yet another method of study of reflex pathways in the spinal cord, to which pharmacological analysis could be applied with profit. At birth the monosynaptic reflex is the only one yet established, and inhibition does not occur until the fifth or sixth day. In these early days of pure monosynaptic excitatory phenomena, the pharmacological picture may well be simpler. Polysynaptic pathways do not begin to develop until the tenth to the fifteenth day and follow the development of dendritic activity.

Finally, a note of caution in the interpretation of ventral root records is sounded by Beswick & Evanson (23), who find that not all motoneurones have effective monosynaptic connections and that, during posttetanic potentiation the ventral root potential may be increased by the inclusion of heteronymous neurones so that a false estimate of the activity of homonymous motoneurones may result.

*Sensory afferent-interneurone synapses.*—Primary afferents in the dorsal roots not only form monosynaptic reflex links with motoneurones but also synapse with interneurones. A good example is the synapse between primary afferents and cells in Clarke's column, which relay impulses to the cerebellum via the dorsolateral tract [Lloyd & McIntyre (174); McIntyre (186)]. McIntrye (187) draws attention to the difference between this type of synapse and the synapse between similar afferents of the stretch reflex with motoneurones, the former showing no posttetanic potentiation, the latter exhibiting this phenomenon to a large degree [Lloyd (172); Eccles & Rall (81)]. McIntyre regards the transmission to cells of Clarke's column as of the "basic" type, having a one-to-one relationship and showing no capacity for "learning."

Another similar synapse probably occurs between primary afferents and cells in the dorsal tracts. The so-called negative intermediary potential is supposed by some workers to be presynaptic, by others to be postsynaptic. Evidence for the latter view has been brought forward by Austin & McCouch (12). Furthermore, Takagi (231) has come to the same conclusion and (232) has carried out a pharmacological analysis of the transmission process. This analysis shows, however, that a wide variety of active chemical substances, including glucose, respiratory enzyme inhibitors, potassium and calcium ions, ACh, and sympathin all have exactly the same effect on the negative intermediary potential. All depress it in high concentration and potentiate it in low concentration. Takagi makes no suggestion about the possible mechanism of transmission but infers that the origin of the potential is physicochemical—an inference that has merit of being, to say the least, undeniable. Howland *et al.* (144) have demonstrated by a microelectrode map that reflex inhibition from stimulation of primary afferents will extend

up the dorsal tracts to the brain stem and that this inhibition is increased by barbiturates.

Other groups of primary afferent-interneurone synapses have been studied by Eccles *et al.* (80), who found proprioceptive afferents of Groups Ia and Ib ending in synapses with interneurones in the intermediate nucleus of the grey matter; and by di Biagio & Grundfest (24) who located the start of the spinoolivary tract at the level of L 5, where there is a group of synapses between fibres of the dorsal tract and cells of the dorsal spinoolivary tract. No activation of the tract could be accomplished by stimulation of trunk afferents.

The cells of primary sensory afferents are located in the dorsal root ganglion, and the axons divide so that collaterals end in synaptic knobs on motoneurones and on interneurones in Clarke's column [McIntyre (187)]. On the assumption that one cell liberates only one transmitter [Fatt (92)], the monosynaptic excitatory transmitter will also be the transmitter at these latter synapses. The failure of Takagi (232) to demonstrate any definite pharmacological specificity is, then, one further indication of our lack of knowledge concerning the nature of the transmitter.

*Interneurone-interneurone synapses.*—A considerable literature is developing on compounds which are supposed to block interneurones and the term "interneuronal blocking agent" is insidiously invading terminology. The basis of the term is that the drugs concerned do not block the monosynaptic reflexes but have a marked depressant action on polysynaptic pathways. It is inferred that, since there is no apparent direct action on the primary afferent or the motoneurone, the blocking action must be exerted on interneurones; but there is no evidence to exclude a blocking action on transmission at primary afferent-interneurone or at interneurone-motoneurone synapses, nor can a direct effect on the interneurones themselves be excluded. Yet the term is used by some authors as if it implied a blocking action at interneurone-interneurone synapses, i.e., in a polysynaptic pathway involving at least four neurones. Feldberg's hypothesis (95) of alternating cholinergic /noncholinergic neurones, implies that the interneurone-interneurone synapse in a trisynaptic pathway is cholinergic. The indirect stimulant action of ACh and anticholinesterases on ventral root potentials (97, 137) can be accounted for by excitation at such a synaptic junction, and there may well be a temptation to visualise the action of other drugs as being exerted on a junction more familiar by reason of being cholinergic.

The first "interneuronal blocking drug" to be fully investigated was mephenesin (17, 130, 150). Mephenesin has little if any blocking action on the neuromuscular junction (17), so that it is unnecessary, even for analogy, to suppose that it acts on a cholinergic interneurone-interneurone synapse as outlined above. Recently, King & Unna (154) have shown by a refined technique (153) that mephenesin blocks the linguomandibular reflex but not the patellar reflex, thus confirming it as an "interneuronal blocking agent." They have also shown that stimulation of the bulbar reticulum in the midline can depress the monosynaptic and facilitate the polysynaptic reflex,

while stimulation of the rostral or lateral part of the reticulum does the reverse. Mephenesin not only blocked the polysynaptic linguomandibular reflex, it also blocked the effects of stimulation of the bulbar reticulum.

Berger (18), Chen (60), and Slater *et al.* (228) have also studied the actions of "interneuronal blocking drugs." Slater *et al.* (228) have demonstrated an antagonism between 5-HT and LSD in their actions on the flexor reflex and have suggested that 5-HT may be a neurohormone, but they have little basis for this contention. Since the interneuronal arc of a polysynaptic reflex may extend into the higher centres of the brain, many of the "interneuronal blocking drugs" may have other actions not directly related to spinal activity. Thus the central actions of mephenesin were reviewed by Berger (17), who has recently (18) described how a similar compound produces flaccid paralysis and prevents strychnine and electric convulsions. The generally sedative effect of these drugs could be held to suggest a blocking action on interneurones in the reticulum where excitation is known to lead to arousal [Arduini & Arduini (9, 10)]. Funderburk & Woodcock (111) have described the sedative properties of milontin, a drug similar to trimethadione, and Bogue & Carrington (26) have described the similar properties of mysoline.

Another group of studies has been devoted to the conditioned reflex, which similarly involves numerous interneurone-interneurone synapses. Bridger & Gantt (32) and Cook *et al.* (62) have examined the actions of drugs on this type of response. Mik'helson *et al.* (195) showed that conditioned reflexes could be blocked by caramiphen hydrochloride (Parpanit), atropine, and scopolamine, this block being prevented by physostigmine or neostigmine (Prostigmine). These data could also be quoted as evidence for the cholinergic nature of some interneurone-interneurone synapses.

*Interneurone-motoneurone synapses.*—There is no obvious distinction between the synaptic knobs on motoneurones which will indicate whether they are terminals of primary afferents or of interneurones, or whether they are excitatory or inhibitory. It is worth noting again, however, the work of Malcolm (191) which could indicate that since the appearance of polysynaptic pathways is correlated with the development of dendritic activity, the synaptic knobs associated with interneurones (polysynaptic pathways) may be more common on dendrites, those concerned with monosynaptic relays being located on the cell body.

Eccles *et al.* (78) have provided evidence for the existence of a cholinergic relay between the collaterals of motoneurones and Renshaw cells and claim that the axons of the Renshaw cells end in inhibitory synaptic knobs on the motoneurones. They have no evidence as to the nature of the inhibitory transmitter, but have produced data to the effect that it is blocked by strychnine. The cholinergic synapse between the Golgi collaterals and the Renshaw cells is blocked by dihydro-$\beta$-erythroidine but not by tubocurarine, and activation of these cells can be produced by ACh or by eserine but not by neostigmine. Fatt (92), in his penetrating review, draws attention to the differences that have been observed in the central nervous system between tubocurarine and dihydro-$\beta$-erythroidine [Wright & Taylor (250)] and be-

tween physostigmine and neostigmine [Schweitzer *et al.* (223)] and concludes that the distinctive pharmacology of this particular junction may be characteristic of other cholinergic junctions in the central nervous system.

The Renshaw cells (217) which are involved in this inhibitory pathway of Golgi collaterals are situated in the ventral horn close to the motoneurones. They probably make up, therefore, a considerable number of the interneurones recorded by Kolmodin & Skoglund (158) in their intracellular recording of interneurones in the ventral horn. Afferent physiological stimuli were used to obtain discharges from these cells, which had resting potentials of some 40 to 80 mv. and spikes of up to 90 mv. which occasionally showed a reversal. Some cells were silent, others had a background discharge at 50 to 60 c.p.s. Afferent stimulation increased the discharge frequency to 200 c.p.s. or abolished it, and the authors have made a classification on functional grounds of some 200 such cells. Cells excited by afferent stimulation could be Renshaw cells; cells inhibited must represent some other group. On the other hand, Kolmodin (157) has shown that some such interneurones mediate a discharge from motoneurones, so that cessation of their discharge could act as an effective inhibitory mechanism.

Kolmodin & Skoglund (158) also showed that an irreversible anticholinesterase induced activity in the interneurones and a decrease in the resting potential; this points to a cholinergic synapse and would fit in well with the hypothesis that the cells concerned were Renshaw cells. Sympathin also influenced the discharge rhythm and in some cases changed the pattern of response so that previously nonactive afferent sources became effective. This might be explained on the basis of a modulating action of sympathin on transmission processes.

*The motoneurone pool.*—The evidence which has accumulated recently would, if confirmed, imply that the motoneurone is under bombardment from primary afferents and certain interneurones which release an excitatory transmitter, and from certain other interneurones, including the interneurones activated by Golgi collaterals, which release an inhibitory transmitter. The level of activity of the motoneurones is determined by the balance between the two influences.

Brock *et al.* (35) have produced evidence that the critical factor in the motoneurone discharge is the level of the resting potential which is decreased by activity of afferents from agonist muscles and increased by activity of afferents from antagonist muscles. There would not appear to be any direct interaction between the two chemical transmitters. Gualtierotti (119) showed a D.C. shift of the ventral root potential during reflex activity and postulated that variation in the D.C. potential was the basis of inhibition and excitation. He has recently (120, 121) studied the actions of caffeine and strychnine in increasing the root potentials, both resting and synaptic. He states that the effect of strychnine, unlike that of caffeine, outlasts the period of reflex activity. These findings can be explained on the basis that strychnine acts by blocking the inhibitory pathway. Brooks *et al.* (39) suggest that tetanus toxin has a similar effect. Further evidence of the importance of the

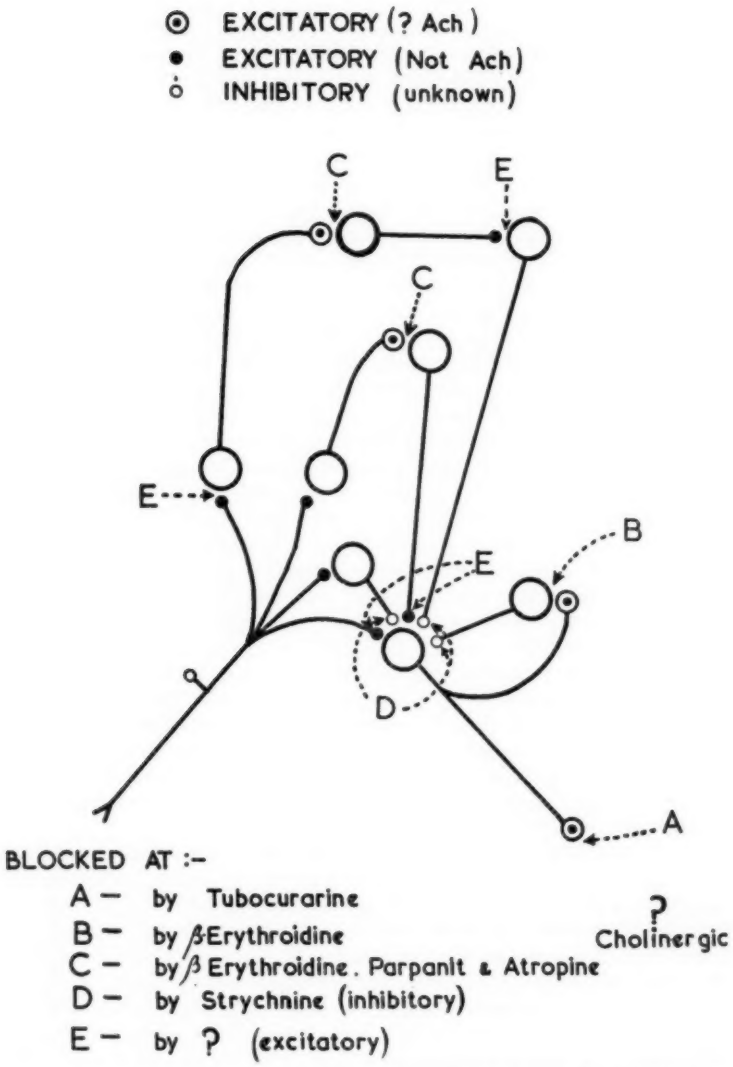


FIG. 1. Diagram of spinal synapses. The term "?Cholinergic" should be linked with synapses B and C.

resting potential level has been adduced by Fuortes (110) who applied a D.C. potential to the ventral root and thus produced repetitive firing of motoneurones. He suggests that rhythmical firing of the motoneurones indicates a steady state of depolarization as well as a rhythmic bombardment of the cells by interneurones.

*Chemical transmission in the spinal cord.*—The evidence described has been summarized in Figure 1. It seems apparent that there must be at least three chemical transmitters acting in the spinal cord, as suggested by Fatt (92). There is good evidence for the excitatory rôle of ACh on certain interneurones; there is equally good evidence that it plays no part at the motoneurone pool, where excitatory and inhibitory transmitters, both unknown, are active. Dihydro-β-erythroidine, Parpanit, and atropine probably block at cholinergic central synapses; strychnine probably blocks at central inhibitory synapses. No drug blocking the central excitatory transmitter at the motoneurone or elsewhere is known.

Nor is the nature of the transmitters themselves in any way clear. Substance P and ATP have both been suggested as the excitatory transmitter to motoneurones, but the evidence for either is slender. Sympathin is probably not directly involved in central transmission.

Nevertheless, much progress has been made in our knowledge of the pattern ot these events; and the recent interest in pharmacological methods of study is encouraging.

## Transmission in the Brain

*The area postrema.*—From an anatomical study of the area postrema, Clemente (61) suggested that it was a receptive area associated with the vascular system. Lying along the lateral border of the caudal end of the fourth ventricle, it contains neuroglia but no neurones, and it was originally noted because of its high permeability to dyes [Wislocki & Putnam (245)], staining readily with trypan blue. Borison & Wang (27) have stated that it contains chemoreceptors which cause vomiting. Vogt (241) found that it contained sympathin in very large amounts which was not released by stimuli which depleted the hypothalamus, and Amin *et al.* (7) reported equally striking amounts of 5HT and substance P. These workers speculated as to the functional significance of the area and suggested that it contained the active principles only because of its chemoreceptive properties. Feldberg & Sherwood (100) in commenting on the effects of drug injections into the lateral ventricle of the cat, mentioned the very high pharmacological sensitivity of the areas round the ventricles and suggested that the cerebrospinal fluid might act as a carrier mechanism for active principles to sensitive areas. They were in fact implicating the structures in the reticular formation as the senstitive areas concerned, but it is quite possible that the area postrema has a part to play in these reactions. It is worth noting that nearly all drugs investigated by Feldberg & Sherwood (100) shared in the property of causing retching, and this relatively nonspecific effect may well be attributed to stimulation of chemoreceptors for vomiting in the area postrema.

These findings are of importance to the present subject only insofar as they illustrate that not all central actions of drugs are necessarily attributable to an effect on transmission.

*The reticular formation.*—Moruzzi & Magoun (196) found that they could mimic the cortical responses to stimulation of the sciatic nerve by stimulating the medial reticulum, and they concluded that there is a common multisynaptic relay station there for ascending fibres; and Purpura (214) has reported a similar finding. There is now considerable evidence (9, 107, 115, 132) that the reticular formation, in addition to the ascending pathways, also contains descending fibres and neurones efferent from the cortex.

Furthermore, there has developed an interest in the pharmacological reactions of the reticulum. Arduini & Arduini (10) noted that the chains of interneurones in the reticulum were depressed by pentobarbital (Nembutal), ether, trimethadione (Tridione), and procaine, and postulated that the block of arousal that resulted might be the cause of the anaesthesia; they also noted that the cells of the reticulum were specifically sensitive to excitants such as strychnine and leptazol (Metrazol). Feldberg *et al.* (98) also describe a specific sensitivity of the reticular cells to the action of tubocurarine injected intraventricularly. Bertrand (22) and Zanchetti & Brookhart (253) in studying the "indirect" pathway from the cortex to the cord, similarly noted an enhancement of the response by strychnine and leptazol respectively, together with a depression by pentobarbitone. It is tempting, from studies on the cord, to speculate that strychnine is exerting its effects by blocking inhibitory neurones in the reticulum.

Ingvar (146, 148) has studied the effects of reticular stimulation on the electroencephalogram (EEG) and on the circulation through the cortex. He has noted that barbiturates depress both effects and is of the opinion that such extraneuronal effects may account for some of the changes following reticular stimulation.

Longo *et al.* (176) have studied the actions of "ganglioplegiques centraux." These compounds, the best known being Parpanit and Largactil, block the EEG response to external stimuli and also block the grand-mal EEG pattern normally evoked by nicotine. The compounds also block transmission in autonomic ganglia, and the authors consider that they owe their central activity to the fact that, as tertiary amines, they can penetrate nerve cell membranes. They speculate on the possiblity that their central action is due to a block of transmission through the reticulum and suggest that there may be cholinergic synapses there.

Feldberg (96) has reviewed the effects of sympathin on the reticular formation, and has drawn attention to the fact that Vogt's findings (241) on the distribution of sympathin, and those of Amin *et al.* (7), indicate that there are high concentrations of both in the reticulum. He noted that the effect of sympathin given intraventricularly was to produce a sort of anaesthetic state, and he speculated on its possibilities as a mediator of sleep, arousal, and stress fatigue (100).

*Cortical synapses.*—Elegant studies of the electrical properties of the

isolated cortical slab [Burns (50)] continue to be made [Burns & Grafstein (53); Burns (51, 42)]; the question of whether the isolated slab is "silent" has also received further attention. Echlin *et al.* (83), Henry *et al.* (131), Kristinsen *et al.* (163) and, most recently, Ingvar (147) stated that they observed spontaneous activity, and Bremer (31) suggested that the size of the slab might be the determining factor. However, Wright (251) has now brought forward evidence suggesting that failure to get a silent slab is entirely dependent upon failure completely to isolate it neurologically. Wright also found that strychnine, applied locally, would not invoke any activity, but that application of strychnine together with electrical stimulation would do so. An afferent input is thus necessary for strychnine convulsions, and this can be readily explained on the assumption that strychnine acts by blocking the inhibitory pathways.

Hendershot & Chenowith (129) have begun to use the isolated cortical slab for pharmacological studies. They describe how fluoroacetate, applied locally, causes an accumulation of citrate and activity of the seizure type. Benitez *et al.* (16) found that fluoroacetate produced an accumulation of ammonium ions.

McIlwain and his co-workers (13, 177, 178, 179, 181) have evolved another intriguing technique for studying the cortical metabolism, using respiration rate as the index of activity. The electrically-stimulated cortex is much more sensitive than the resting cortex, although doubts in interpretation arise since it is not certain whether the neurones or the glial cells are activated by such stimulation. In a recent paper, Lewis & McIlwain (171) reported that ergot derivatives (including LSD) and *N*-2(-chloroethyl) dibenzylamine (Dibenamine) inhibit respiration and glycolysis. No antagonism could be found between epinephrine and Dibenamine or between LSD and 5-HT. Earlier work demonstrated inhibition by anticholinesterases (105) and veratrine (180, 247).

*Behavioural changes.*—Himwich and his colleagues (8, 134) and Harwood (125) have studied the effects of injection of DFP into the carotid artery in rabbits. This is followed by a peculiar behavioural state in which there is forced circling of the animal in one direction accompanied by reduction of ChE in the frontal cortex of the opposite side. Atropine, scopolamine, or Parpanit will prevent the syndrome, which the authors think is attributable to ACh accumulation. Once circling is established, its direction can be reversed by giving dextro amphetamine (Dexedrine), caffeine, or atropine in large doses; small doses of atropine are effective if given together with strychnine.

Feldberg & Sherwood (99, 100, 101) have studied the effect of a large number of drugs given by intraventricular injection. Some of the possible implications have already been discussed. It is worth noting here, however, that DFP, physostigmine, and large doses of ACh induce a condition closely resembling catatonia (101). The authors consider that this syndrome is attributable to the accumulation of ACh near the structures around the ventricle. Sherwood (226) has treated schizophrenic patients with intra-

ventricular cholinesterase and has obtained some remissions. De Jong (149) showed that bulbocapnine could produce the same syndrome, and recently Pappalardo & Lipani (199) showed that this compound would increase the ChE activity in the basal ganglia and cerebellum, while depressing it in the cortex and brain stem. Feldberg & Sherwood (102) found that bulbocapnine, in extremely small doses intraventricularly produced an almost immediate onset of catatonia and of changes of "personality." These effects were very similar to those described by Hess (133) after electrical stimulation of the diencephalon or after production of lesions above the mammillary bodies rostral to the red nuclei. These findings are strikingly similar to those reported by Feldberg & Sherwood for anticholinesterase and ACh injections intraventricularly.

Feldberg & Sherwood (101) also point out that Bornstein (28) described a high concentration of ACh in the CSF in concussion and infer that this form of stupor may have a similar etiology. Feldberg & Sherwood (100) also find that atropine and tubocurarine produce opposite effects to ACh, i.e. they are stimulants (tubocurarine even producing a syndrome resembling epilepsy). They note the anaesthetic effect of sympathin and suggest that stress fatigue may have a similar origin; they also observe that the itching seen after anticholinesterase may be related to that which occurs after stimulation of the septum lucidum and the area above and behind the mammillary bodies [(Hess (133)].

These extremely interesting observations are, at present, hard to interpret. It is tempting to speculate that, for example, sympathin exerts its effects on chemoreceptors in, say, the area postrema, and that the retching common after most drugs given by this route is attributable to a less specific stimulation of chemoreceptors for vomiting in the same region. It may well be, however, that some of the other actions, especially of ACh and the anticholinesterases result from a direct action on cholinergic neurones in the vicinity.

## Synapses in Autonomic Ganglia

The evidence for the existence of cholinergic synapses in the sympathetic ganglion is now virtually complete. The ganglion is exceptionally rich in ACh (183), and it also contains ChA (93) and ChE (222). Stimulation of the presynaptic fibres releases ACh [Brown & Feldberg (41); MacIntosh (182); Perry (206)]; and injected ACh produces depolarization of the ganglion cell membrane [Paton & Perry (202)] and repetitive firing of the postsynaptic fibres [Brink *et al.* (34)]. Similar evidence has been produced for the parasympathetic ganglia in a more indirect fashion. The only localised aggregates of parasympathetic ganglion cells are to be found in the ciliary, otic, and sphenopalatine ganglia and of these only the ciliary is readily accessible. It contains a lot of ACh (183), and injected ACh will produce depolarization of the ganglion cell membrane and potentiation of a submaximal postsynaptic spike [Perry & Talesnik (211)]. On the other hand, the release of ACh on stimulation of preganglionic fibres was difficult to show since the postsynaptic fibres are also cholinergic, but Emmelin &

Muren (86) were able to demonstrate a presynaptic release of ACh in their experiments on the salivary glands. Holaday *et al.* (135) have recently shown that the effects of DFP on sympathetic ganglia are all consistent with the hypothesis that the facilitation observed is due to inactivation of ChE at cholinergic synapses. DFP was shown to have no consistent effects on presynaptic conduction.

Many problems associated with transmission in the ganglion might be elucidated by the use of intracellular recording with microelectrodes, and recently three partially successful attempts have been made to accomplish this [Eccles (82); Malcolm & Perry (192); Pascoe (200)]. Pascoe used an ingenious device for the introduction of the microelectrode, mounting it on a piezoelectric crystal and moving it forward by passing a direct current pulse through the crystal. Malcolm & Perry (192) were able to facilitate a penetration by digestion of the ganglion with a mixture of hyaluronidase and trypsin, which did not cause any detectable interruption of transmission.

There are many peculiar factors about the records obtained from ganglion cells impaled with a microelectrode. None of the groups of workers ever succeeded in obtaining a response of the cells when using antidromic stimulation, and it appears that there must be a low safety factor for transmission from axon to soma; furthermore, although both Pascoe (200) and Eccles (82) found small synaptic potentials (5 to 10 mv.) in response to orthodromic volleys, it was only very rarely that either was able to record a spike discharge of the cell. The resting potential observed varied from 20 to 90 mv., with a mean from 30 to 40 mv.; the spike discharge has occasionally been observed to overshoot [Eccles (82)]. Failure to obtain transmission might be attributable to damaging of the cells during the introduction of the microelectrodes; on the other hand, Malcolm & Perry (192), who obtained consistently high values for the resting potential, were unable to obtain spike discharges even with orthodromic volleys; but this may alternatively be attributed to the effect of enzymic digestion having caused a separation of boutons terminaux from the cell surface. In the opinion of the reviewer, however, it is also possible that there is no real conduction in the ganglion cell but that conducted impulses are generated at the axon hillock or distal thereto. Failure to record, with a microelectrode in the cell, the spike activity in the axon could possibly occur because of the volume conductor characteristics of the ganglion, although Diamond *et al.* (69) did pick up transmitted spikes in the axons of afferent fibres when recording with a microelectrode in the Pacinian corpuscles leading to them. The occasional spike discharges observed by both Eccles (82) and Pascoe (200) could then be attributed to a chance penetration of axon or of axon hillock. A similar situation is said to occur in the neurones of the lateral geniculate nucleus [Bishop & McLeod (25)] and in the Pacinian corpuscles [(Diamond *et al.* (69)]. The observations of Paton & Perry (201, 202) in the analysis of the ganglion action potential could be explained on the basis of a local response of the cells and a spike discharge in the axons, and Eccles' (73) classical results can also be reinterpreted on this basis. Eccles claimed that the slow potentials recorded from

the ganglion decayed with a measurable space constant, whereas the $S_2$ spike was propagated without decrement. Closer analysis of his published records shows [Paton & Perry (203)] that the $S_2$ spike also decays by some 50 per cent and that the space constant of that fraction, which does so decay, is identical with that of the slow potentials. Yet Eccles (74) was able to produce ganglion responses with antidromic volleys, in contrast to the results with intracellular electrodes; and Laporte & Lorente de No (166) concluded that the antidromic volley, propagated over the cell body, was the reason for the transmission block which enabled them to record synaptic potentials in response to a following orthodromic volley. Further elucidation of the process must await the development of an even more adequate technique for the intracellular introduction of microelectrodes.

In spite of the weight of evidence for the existence of cholinergic synapses in the sympathetic ganglion, suggestive signs of the rôle of another active substance and of the possibility of the existence of a separate group of cells activated by some other mechanism continue to be found. Shaw (225) invoked this as a possible cause of the differential activity he observed when using a variety of ganglion blocking drugs; and similar patterns were noted by Ambache & Robertson (6) and by Ambache *et al.* (5) in denervated preparations. The latter workers also observed a nicotinic action of natural muscarine. Ambache (4a) found that botulinus toxin, injected directly into the ganglion, blocked the fibres supplying the nictitating membrane much more readily than those supplying the pupil, and concluded that there might be two types of synapse in the ganglion. The possibility remains, however, that the site of injection may have been the important factor in this work, although the author does not believe this to be so.

Kewitz & Reinert (152) noted the failure of the ganglion to react to ACh while it still reacted to presynaptic stimulation, when the ionic composition of the perfusion fluid was changed, and speculated on the possible existence of noncholinergic synapses in the ganglion.

Perry & Reinert (209, 210) have extended these observations and those of Ambache and his colleagues (5, 6). They observed the failure of ganglion-blocking drugs to block ACh while still blocking the effects of presynaptic stimulation when the potassium ion concentration in the perfusion fluid was reduced; and they suggested a tentative explanation which does not require the participation of such an alternative transmission process.

*Acetylcholine release.*—The amount of ACh released from a perfused ganglion is usually measured by perfusing with an anticholinesterase present, and, under such conditions as these, the amount liberated during continuous stimulation falls exponentially (41, 182, 206). Perry (206) obtained evidence that, in the absence of an anticholinesterase, this fall does not take place, or, if it does, it is much reduced, so that the safety factor for transmission remains high. This is offered as an explanation of why, under the influence of an anticholinesterase, the ganglion fatigues, unless the rate of stimulation is low. Brown (40) has recently demonstrated how great the safety factor for transmission in the ganglion really is, by observing that at 20°C. an isolated ganglion transmits perfectly well but releases only about 10

per cent of the amount of ACh it releases at 39°C. This observation agrees with the finding that in the physostigminized ganglion the output of ACh falls to some 5 per cent of its original value, while transmission may continue apparently unaffected.

Brink (33) in reviewing the rôle of calcium in synaptic transmission, came to the conclusion that it acts wholly presynaptically. In low calcium solutions, there is a block of ATP and ChA, and a fall in the amount of ACh released. Hutter & Kostial (145) found that raising the calcium concentration increased the amount of ACh released by stimulation, and that this action of calcium was antagonised by magnesium. Similar results were obtained by del Castillo & Engbaeck (55) for the neuromuscular junction; and del Castillo & Katz (56), while they agree with Brink (33) in regarding the action of calcium as a presynaptic one, consider that it is not the result of ChA inhibition but is attributable to a depression of the presynaptic terminals, thus reducing the number of units in action.

*Axotomy.*—Brown & Pascoe (42) noted a failure of transmission through the inferior mesenteric ganglion after section of the postsynaptic fibres (axotomy), although conduction was unimpaired and ACh release appeared to be normal. Transmission improved after a rest period and was interrupted by tetanic stimulation, and McLennan (189) found that the "true" ChE content in axotomised ganglia fell by about 50 per cent. This led to a suspicion that transmission was failing because of accumulation of ACh; yet treatment with tubocurarine failed to relieve the block. McLennan (189) also studied the ChA content of normal and axotomised ganglia and found no difference; however, when potassium was added in these *in vitro* experiments, synthesis of ACh in the normal ganglion was increased three- to ninefold but no such enhancement was found in axotomised preparations. Neither the ChE nor the ChA changes can easily be held to account for the transmission failure.

Brown & Pascoe (42) also noted that, although there was complete failure of transmission *in vitro*, there always remained, after axotomy, a small transmitted response when the ganglion was tested *in vivo*. This they considered might be due to regenerating axons; the alternative explanation, namely, the presence in the ganglion of some noncholinergic synapses which were not affected by axotomy, they ignored. Acheson & Remolina (2) have obtained results essentially in agreement with those of Brown & Pascoe (42); they suggest, on the other hand, that the transmission failure may be similar to that attributed by Downman *et al.* (70) in the spinal cord to mechanical disruption of the synaptic knob-cell membrane junction because of swelling of the cell body after axotomy. Brown & Pascoe (42) reject this explanation as invalid in the case of the ganglion since they have evidence of a loss of sensitivity of the ganglion cells to injected ACh.

*Denervation.*—After section of the presynaptic fibres the ganglion loses much of its ACh [MacIntoch (182)], ChA [Feldberg (93); Banister & Scrase (15)], and true ChE [Sawyer & Hollinshead (222)]; it also becomes more sensitive to injected ACh [Cannon & Rosenblueth (54)]. The fall in the ChE content has often been assumed to be the reason for this increased sensitivity

although Emmelin (85a) has evidence that this is not true in parasympathetic ganglion cells. Perry & Reinert (209, 210) have, however, recently studied other changes in the ganglion which follow presynaptic section. The methonium drugs which block the normal ganglion without depolarizing the cell membrane [Paton & Perry (202)] may, in the denervated preparation, excite the ganglion cells, and always fail to block the stimulating action of ACh. It is difficult to account for such changes on the basis of an altered ChE content. Moreover, similar changes are observed in the normal ganglion when it is perfused with a low potassium saline [Perry & Reinert (209)] or under anaerobic conditions [Reinert (216)]. All of these changes can be reversed by the addition of suitable concentrations of glutamate or aspartate to the perfusion fluids (209, 210, 216). The altered responses of the normal ganglion to injected ACh are not accompanied by similar alterations in the response to presynaptic stimulation.

Perry & Reinert (210) believe that most of the changes can be attributed to an alteration of the metabolism of the ganglion cells such that, in each case, the cells can no longer metabolise glucose aerobically. In Reinert's (216) experiments, this has been accomplished directly by removing oxygen from the milieu of the cells. In the other experiments Perry & Reinert (209) suggest that a reduced potassium concentration inhibits aerobic glycolysis since the acetate-activating enzyme is known to be potassium-dependent. Gertner & Reinert (117) have shown that the potassium content of a ganglion falls after denervation or after perfusion with potassium-free solutions and that it can be restored by perfusion with glutamate or aspartate.

Perry & Reinert (210) also suggest that the blocking activity of the methonium compounds is attributable to an antimetabolic effect at some point in the chain of aerobic glycolysis; their failure to block ACh in the denervated preparation is attributed to the fact that this preparation is obtaining energy only by anaerobic glycolysis. The inference from this work is that, if confirmed, it indicates that "transmission block" may not be the competition with ACh receptors that is commonly visualised but a direct action on the metabolic activity of the cell, resulting, one presumes, in a failure of the cell to generate sufficient energy to fire a propagated response in the axon. If this is true, the concept of competitive inhibitors will require some modification; and some of the anomalies of structure-action relationships may become more comprehensible.

Larrabee *et al.* (167) found that, when glucose was present, an excised ganglion continued to transmit as long as did an acutely denervated ganglion *in situ*, but in the absence of glucose transmission failed quite rapidly, especially if the ganglion was stimulated. Perry & Reinert (210) found, however, that the chronically denervated ganglion, when excised and kept without glucose, continued to react to ACh for much longer than did the normal ganglion. Horowicz & Larrabee (143) found that failure of transmission in the absence of glucose was accompanied by a failure of conduction in the C fibres, which may also result from a failure of energy production. Oomura & Hashimura (198) found that compounds participating in the Krebs cycle decreased the sensitivity of nerve at the nodes of Ranvier. Koshtojanc (162),

in reviewing recent work on this subject, described the block of ganglion transmission produced by cadmium chloride and suggested that this was caused by a blocking of sulphydryl groups and could be reversed by cysteine. It was considered that block of sulphydryl groups would prevent the release of ACh.

Konzett & Rothlin (161), following up the idea that the increased sensitivity of a denervated ganglion might be attributable to a lack of exposure of the cells to the transmitter, attempted to mimic the effects of denervation by prolonged block with hexamethonium and completely failed. The experiment is not conclusive, however, in that hexamethonium prevents only the effects and not the release of the chemical transmitter.

*Potentiation of the action of acetylcholine.*—The rôle of sympathin in the ganglion has already been discussed. Konzett (159) described how small doses of histamine could sensitize the ganglion cells to the action of ACh but rarely themselves caused excitation of the ganglion cells. Trendelenburg (237, 238) has studied these actions of histamine and finds, however, that histamine will regularly potentiate the effect of submaximal stimulation. This action of histamine is blocked by mepyramine, cocaine and nicotine, but not by hexamethonium or atropine. Pilocarpine has a similar action and is blocked by atropine but not by mepyramine. The explanation of these findings is obscure.

Konzett & Rothlin (160) described a sensitization of the ganglion cells produced by ouabain and other cardiac glycosides. This has been confirmed by Perry & Reinert (208); the explanation of this action is also obscure, but it is not found in the absence of potassium and consequently may be related to the metabolic activity of the ganglion cells.

*Ganglion-blocking compounds.*—There has been continued work on compounds which possess ganglion-blocking activity; no attempt has been made to review most of these papers, since they do not assist an understanding of the physiological basis of synaptic transmission. It is worth mentioning, however, that Gertner (116) has demonstrated activity of this kind for Compound 48/80, which is widely assumed to be a specific histamine liberator and is used as such. The block produced by 48/80 does not appear to have anything to do with its power of releasing histamine, nor is it due to interference with the release of ACh. Gertner assumes the action to be a competition with ACh. Exley (90) has confirmed that barbiturates have a considerable ganglion-blocking activity, and this must be remembered when barbiturates are used as anaesthetics in experiments on ganglia.

Limitation of space has prevented discussion of a number of papers which have been published and which may be of interest to the reader. Among them are the following: Armin *et al.* (11); Burn *et al.* (48, 49); Chatfield & Purpura (59); Cragg (63); Crossland and Mitchell (65); Davies (67); Delgado (68); Eccles (76); Edge (84); French *et al.* (106, 108); Ginzel *et al.* (118); Gualtierotti (122); Harris & Borison (124); Leusen (169, 170); McNamara *et al.* (190); Perry & Wilson (212); Radouco-Thomas *et al.* (215); Savage & Hard (221); Soulairac & Soulairac (229); and Wien (244).

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# SOMATIC FUNCTIONS OF THE NERVOUS SYSTEM[1,2]

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Fresh concepts on the organization of the central nervous system have emerged from new experimental findings presented in two recent books. Twenty-five years of investigations, with electronic technics, on *Receptors and Sensory Perception*, have lead Granit (165) to emphasize that the only way to a real understanding of central organization is to analyse sensory messages and to follow their destiny through the central complexities: " . . . the brain, after all, is the great interpreter of our senses." A symposium entitled *Brain Mechanisms and Consciousness* (47), which includes work done since the pioneer observations of Bremer in 1935, stresses the fundamental rôle of the reticular systems, individualised by Magoun, and especially their relationship to sleep, wakefulness, and states of consciousness.

A few general statements can be made: (*a*) To the well-known "specific systems" (such as the great sensory and motor pathways and the classical reflex arcs) which are composed of chains of few neurones whose synapses are endowed with a comparatively high "transmitter potentiality" (239), which are strictly organized, and which are relatively insensible to metabolites and anesthetics, are contrasted the "nonspecific systems" (such as the diffuse reticular formations), which are built up of a crowded network of interneurones where the type and local signs of sensory input are lost, which are highly sensitive to metabolites and anesthetics, and the essential rôle of which is to control the activity level of the specific systems. (*b*) The idea of a vertical organization of the neuraxis, where the great sensory and motor paths border on the central core of grey reticular matter, is comparable with the classical horizontal subdivision of the nervous system based on the evolutionary theories of levels of organization. (*c*) The nonspecific brain-stem reticular system belongs to the visceral as well as to the somatic spheres of activity, it being affected by exteroceptive and interoceptive afferent impulses and its continuous tonic activity depending directly on crucial components of the milieu intérieur; at the same time it controls sensory receptors and motor effectors, as well as visceral regulatory efferents. Such a conception restores the unity of the central nervous system, lost since Bichat's classification. These ideas, now clearly recognised, have determined the plan of presentation in this and previous reviews (55, 116, 238).

[1] This review is based on papers available in Europe from July, 1954 until the beginning of June, 1955.

[2] The following abbreviations are used in this review: ACh (acetylcholine); ACTH (adrenocorticotropic hormone); EEG (electroencephalogram); ERG (electroretinogram); RF (reticular formation).

[3] We are indebted to Dr. Ian Macdonald, of London, for his generous help in the writing of this paper.

*Books and general studies.*—Up-to-date information in English may be found in Fulton's *Textbook of Physiology* (149), which includes many rewritten sections, one being a clear account by Lloyd on the "Principles of Nervous Activity." In French, Morin (257) has recast his texbook for students, while in German, Jung (206, 207) has surveyed basic neurophysiology and its technics. The long-awaited re-edition of Cajal's work has now appeared (70). Hess has produced an enlarged edition of his *Zwischenhirn* (177) with an English translation (178). Neurohumoral transmission (250, 268) has been discussed, as has neurosecretion (27, 311). Additional works include publications on neurosurgical and psychosomatic approaches (127, 230, 248, 282, 300, 325), together with general discussions on central nervous regulatory functions (345, 354). The development of the concept of reflex activity is covered by a critical study (74), Descartes's historical contributions (120), and comments on the latter (326).

## SPECIFIC AFFERENT SYSTEMS

The physiological bases of perception have been expressed by Adrian (in 47). Granit has extensively discussed retinal and muscle spindles activities as well as the centrifugal control of sensory receptors. He has stated the fundamental principles in his *Receptors and Sensory Perception* (165). The main recent data on sensory functions may be found in the chapters written by Ruch and by Patton in Fulton's textbook (149). A complete report (with 506 references) on Soviet researches on sensory interaction is available (240).

### Somesthesis

The whole field of somesthesis has been recently surveyed by Weddell (361).

*The receptor level.*—A critical review of the controversial literature on the structure and function of mammalian cutaneous nerve endings makes clear that the old concept of four distinct sensory modalities subserved by morphologically specific nerve endings must be seriously re-examined (Weddell *et al.* (365). Recent results obtained with artefact-free methods support the view that the function of the sensory fibers is determined not so much by their neurohistological configuration as by their cutaneous locality, by their distance from the surface of the skin, and by their location relative to the stem fiber from which they arise (363). Histological findings on the sensory innervation of the rabbit ear have been discussed in relation to the distribution of hairs and hair follicles (362, 364). Several interesting anatomical papers deal with the sensory innervation of mucous membranes (2, 3, 152). Quantitative relationships between thermal threshold, skin temperature, heat transfer (232), and the cross-sectional area of the stimulus (231) have been investigated. In man the threshold to warmth appears highly variable when measured with radiant stimuli (370). In the frog, deprived of thermal sensitivity, thermal stimulation of the skin or tongue gives rise to nociceptive impulses in the fibers of the dorsocutaneous and glossopharyngeal nerves; in

the medullated A fibers the impulses are only of a "phasic" nature, whereas sustained discharges are recorded from the nonmedullated C fibers (122). In the cat, both pressure and thermal stimulation may induce nociceptive discharges in the thin medullated A fibers (121). In the rabbit's incisor nerve, slow and rapid adapting, as well as spontaneous discharging types of responses, related to the direction of the pressure applied on the dental crown, have been differentiated (267). Action potentials have been evoked in the frog's dorsocutaneous nerve in response to a superficial vibratory stimulus (211).

Only the mechanoreceptors related to joint sensivity will now be considered.[4] In man the sensation both of passive movements and of position of the great toe arises mainly in the region of the joint capsule (59). Nerve endings present in the medial ligament of the knee joint contribute to a proprioceptive inflow carried by large medullated fibers, quite independently of the tone of the muscles acting on the joint (18); a "lamellated" type of receptor is responsible for the slow adapting discharges and a "spray" type for the rapid adapting ones (45). Analogous receptors are located at the epiglottic joint (19).

*Spinal and thalamic levels.*—Before they reach the spinal cord, afferent impulses are delayed in the spinal ganglion at the bifurcation of the sensory fiber (126). Many attempts to identify the spinal somesthesic pathways prove that cutaneous messages can ascend the spinal cord and reach the thalamus by a variety of routes (92, 154, 254, 255) and that it is difficult to correlate individual spinal pathways with individual groups of thalamic neurones; however, no responses other than tactile seem to be mediated by the dorsal columns (156). Spinal relays and conduction velocity of hind limb afferents in the spino-olivary tract (41), as well as the fiber patterns in the anterior and lateral funiculi (especially the dispersion of the spinothalamic tract) (349), have been investigated. An anatomical study has revealed that, in the cat, the fibers previously described as the trigeminothalamic tract are actually fibers of the reticulothalamic pathway (307). The conditions for regeneration of intraspinal nerve fibers have been studied in the adult cat (89). Within the specific thalamic nuclei, Gaze & Gordon have differentiated three types of single neural units specifically responsive to the activation of $\alpha$, $\beta$, or $\gamma$ fibers, $\delta$ fibers, and C fibers respectively. They have confirmed the general arrangement of the tactile thalamic representation, but found a substantial overlap between the regions for the face, fore and hind limbs, as well as an undeniable ipsilateral representation of the hind limb (155).

*Cortical level.*—Krieg has re-examined the architecture of the postcentral gyrus of the monkey and identified the cortical projections of the different parts of the sensory thalamic nuclei (222). Adey, Porter & Carter, by stimulating deep structures, have mapped out the cortical representation of cer-

[4] Work related to muscle spindles and Golgi tendon organs will be discussed under "Reflex Spinal Activity."

tain forms of deep sensibility. The responsive area is more extensive than the classical tactile area but discloses the same general arrangement. There is a high degree of spatial overlap between responses evoked by stimulation of widely-separated peripheral points, but the authors stress the fact that within a responsive area the position of the cortical point yielding the most rapid responses to stimulation of a definite peripheral point remains constant. They suggest that perception of deep sensation may rest in the patterns and sequences of the afferent impulses rather than on their spatial dispersion (4).

During the functional maturation of the cortex, evoked responses can be recorded before any manifestation of spontaneous electrical activity of the cortex (316). In the conscious rabbit the amplitude of the cortically evoked potential is subject to considerable variations related to the level of cortical activity and is followed by a long-lasting depression (284). The microelectrode technique reveals that the pyramidal cell takes part in the somesthesic response and also that the intraneuronal response differs considerably from the classical evoked potential: the early deflection becomes complex and is frequently surcharged by a train of spikes, while the late deflection becomes the most important element of the response (9). The cutaneous field from which a cortical cell may be activated varies from a few hairs to the whole surface of the hand (346). Somesthesic responses have been recorded and analysed outside the classical sensory somatic cortex in a variety of cortical areas such as the gyrus marginalis and the parietal and frontal areas (10), parasensory areas (67), a recently described polysensory area in the mesial cortex (135), and the parietotemporal associative cortex (271).

*Pain.*—Bremer has summarised the main histological, anatomical, and physiological correlates of the pain "phenomenon" and has suggested the possibility of a central facilitory participation of nonnociceptive impulses in the elaboration of pain sensation as well as an explanation of the genesis of hyperalgesia by processes (irritative, etc.) transforming into repetitive discharges the normally noniterative responses of nervous elements (50). In a clinical and surgical report, Bailey *et al.* have given a detailed description of the central trigeminal pathways available for painful stimuli (25). On the basis of histological findings it is highly probable that gastroduodenal pain is mediated by cerebrospinal fibers present in the stomach and the duodenum (272, 273). Hypotheses on the rôle of hypothalamic (329) and thalamic (253) formations in the central mechanisms of pain have been proposed. Typical patterns of the motor and sympathetic responses to pain have been evoked by stimulating brain stem and rhinencephalic structures (114).

## Hearing

Rosenblith has outlined his statistical point of view on the electrical activity of the auditory afferent system (301). Békésy has retraced the steps of his investigations on the mechanical properties of the organ of Corti and his recent results concerning the vibration pattern of the cochlea partition (35), while Davis has reviewed the question of the excitation of nerve im-

pulses in the cochlea (113). The most recent exploration of the complex cochlear potentials indicates that the source of the A.C. (microphonic) potentials is at the hair-bearing end of the hair cells (339) and that of the two components of the D.C. "summating" potential only the first one ($SP_1$) is of neural origin (163). The classical arrangement of the frequency responses of the cochlea has been confirmed by experiments in which very small lesions of the cochlea of the cat were followed by correspondingly very restricted losses in auditory sensivity (333). The ear of the kangaroo rat, the structural peculiarities of which are well-suited for recording the electrical responses of both auditory and vestibular organs, has been explored (210). In the cochlear nucleus of the guinea pig, Tasaki & Davis, repeating with a finer technique (submicroscopic microelectrodes) the older observations of Galambos & Davis, have found spontaneous discharging elements the frequency-responses of which differ from the responses of the elements previously described (338).

At the cortical level, the auditory areas, as determined by "Laplacian" recording of evoked responses, are more limited and sharply-defined than the areas mapped by usual recording methods, their localisation agreeing well with the cytoarchitectural subdivisions of this cortex (283). Bremer *et al.* have analysed the responses of the inferior portion of the ectosylvian gyrus, the characteristics of which are those of a "secondary" rather than of a "second" psychosensory area. This area is normally activated by impulses relayed from the first auditory area, but in certain conditions impulses relayed from subcortical formations are detectable (52). In the first auditory area, while many units have been found responsive to any auditory stimulus, others are activated by clicks, tones, or both (112). In the human cortex triphasic evoked auditory responses have been found widely distributed over the scalp and are maximal at the vertex; these responses disappear in deep barbiturate anesthesia. Auditory stimuli may also give rise to a short train of $\beta$ waves, and this sort of response, but not the usual evoked potential response, is susceptible of conditioning (1). Experimental evidence for a central control of the auditory input, originating in the superior olive region, has been given (153).

### Vestibular Function

The main anatomical and physiological data on the labyrinth and associated structures may be found in the introduction to a clinical paper on vestibular function tests (266). The microphonic responses of the fish lateral line and the cochlea of birds and mammals have been compared, using two pure tones as stimuli (353). At the central level, recording the discharges of isolated neurones in the vestibular nuclei in response to both slow and rapid rotation has shown that both the nucleus triangularis and Deiters' nucleus respond to horizontal rotation and that the post-rotatory activity of the nuclei is characterised by a rhythmic "sawtooth" progressive decrease of the discharges (132). In the cat the cortical projection area of the vestibular

nerve, as outlined by the evoked potential method and Tunturi technique, includes the middle anterior bank of the anterior ectosylvian gyrus and a narrow strip of the posterior bank of the anterior suprasylvian gyrus. Within this area a specific arrangement of the responses with respect to the peripheral branches of the vestibular nerve has been found (16).

## Vision

Antidromic potentials have been evoked in retinal ganglion cells and following this antidromic discharge the excitability, tested by a photic stimulus, is depressed for 100, 170, and 250 msec. for red, green, and blue lights respectively (264). In an extensive analysis of electrical responses from visual central pathways in nonmammalian vertebrates, Buser has shown that intraretinal neuronal mechanisms are largely responsible for the complexity and long duration of the visual potentials recorded at more central levels (66). Below a certain minimal area of stimulated retina the electroretinogram (ERG) differs from the high intensity global ERG (368). Such "area effect" does not influence the maximal fusion frequency since comparable values have been obtained from a single ganglion and the total ERG. In the cat, cone activity takes part in the response to flicker (123).

For the subterranean mole the connections of the superior colliculus to visual and nonvisual centers are as rich as those in other mammals despite the great reduction of the visual inflow (205). For birds an uncrossed visual pathway to the tectum opticum has been demonstrated by the evoked potential method (66) and a correlation has been established between the patterns of the electrical responses and the histological structure of the different cellular layers (105). In the frog and cat, the afferent and efferent connections of the superior colliculus have been explored by electronic methods in relation to visual functions (17). In fishes a somatotopic tectal representation of the retinal quadrants has been demonstrated (66).

A quantitative analysis of the neurones in the layers of the visual cortex in accordance with the distribution of their axons and dendrites has been presented by Sholl together with a statistical approach to the organisation of this cortex (324). Jung has postulated the existence in the visual cortex of a homeostatic system (209) based on a differential synaptic organisation demonstrated by electrophysiological methods (32). In birds visual projections have been found distributed in the frontoparietal region, the occipital area remaining almost silent (306). In the smooth brain marmoset the cortical field responsive to photic stimulation is more extensive cytoarchitecturally than in *Maccaca mulatta* (369). Bremer has demonstrated a new mechanism which may play an important rôle in the higher visual functions, that is the duplication of visual cortical responses by the interhemispheric transfer of the sensory message, without alteration of the temporospatial pattern of response (51). Like the commissural transfer described by Bremer & Terzuolo for other sensory modalities (53), the visual callosal projection is homotopic. The pathways mediating metrazol-induced irradiation of visual impulses have been explored in the cat (192).

### Olfaction

The present state of our knowledge on olfactory function has been expressed in a series of general articles where the problem of olfactory discrimination is discussed from different points of view (5, 34, 246, 265). Indirect information has been obtained on the nature and modality of the olfactory signals: receptor potentials are probably the origin of the potential oscillations recorded directly from the olfactory epithelium (6) as well as of the sustained potentials which can be led off from the olfactory bulb and fibers during olfactory stimulation (274). The possibility of a wide-spread interaction of the signals in the afferent fibers before they reach central structures is suggested by the highly synchronised activity recorded by Adrian in the nonmedullated fibers of the vomeronasal nerve (7). In the olfactory bulb three groups of neurons have been differentiated by their discharge patterns. Of the three groups, the smallest is the only one in which the discharges are altered by olfactory stimulation (356). Effects of this stimulation on the spontaneous activity of specific and nonspecific rhinencephalic formations have been compared: the localized activation of the amygdala and piriform lobe is strikingly different from the high frequency, hypersynchronised activation of the olfactory bulb. In the hippocampus the responses are identical to those evoked by gustative or nociceptive stimuli (164). A comparative anatomical study has shown that the secondary olfactory areas of the human brain occupy a very similar position to those found in the monkey (13).

### Taste

Beidler has proposed a theory of taste stimulation in which he postulates as the initial reaction a loose combination of the ions of the chemical stimulus with some substance of the taste receptors, this reaction leading to the stimulation of the receptor and to the eventual depolarisation of the sensory nerve endings (33). In mammals evidence for a "water taste" and for nerve endings specifically sensitive to water is given by the increased impulse activity recorded in the chorda tympani when the tongue is irrigated with distilled water (235). At the cortical level, the projection area of the glossopharyngeal and chorda tympani nerves has been delimited by the evoked potential method, and the functional rôle of this area has been confirmed by ablation experiments (37). The complex peripheral and central mechanisms involved in alimentary behavior have been investigated by Le Magnen (233).

## SPECIFIC EFFERENT SYSTEMS

### Reflex Spinal Activity

The basic notions have been very clearly stated by Lloyd (in 149). Granit has discussed in detail the functional organization of the proprioceptive system, the efferent control of muscle spindles by $\gamma$ fibers, and the complexity of the interplay of these with the $\alpha$ system (165).

*Stretch reflex and myotatic unit.*—The afferent fibers from the muscle spindles show a bimodal diameter distribution: a fraction of them (originating in the primary, annulospiral or nuclear bag endings) labelled IA belong to the fiber group diameter range above 12 $\mu$ and the remainder (originating in the secondary, flower-spray, or myotube endings) constitute the most substantial part of the 4 to 12 $\mu$ fibers labelled group II. The afferent fibers of the Golgi tendon organs labelled IB have a simple one-peak diameter distribution and account for the rest of the 12 to 20 $\mu$ group (190). Histological and electrical studies have demonstrated stretch receptors in the tongue (96, 97, 229) and in the extraocular musculature (98, 101, 102). In addition to the previous findings of Katz, Kuffler has demonstrated in the crayfish that sensory discharges from the muscle spindles are mediated by a local depolarization of the sensory nerve endings (223). Consequently it is possible to influence the frequency of the sensory impulses by changing electrotonically the membrane potential of the nerve terminals (133).

In the physiology of the reflex activity of the myotatic unit the first problem is that of the intraspinal connections which allow the transmission of sensory impulses from one component of a muscle group to the motoneurones of synergic components of the unit, the so-called "heteronymous" transmission. Cohen has postulated for this transmission two distinct types of spinal connections, namely, strictly localized low-threshold pathways and more diffusely connected high-threshold pathways, the passage from one to the other occurring in a step-like manner (91). Another possibility, favored by Lloyd *et al.* is that any given afferent fiber could be connected with all the motoneurones of the synergic unit and that the "transmitter potentiality" would vary greatly at its several junctions with the different motoneurones, although in a graded, quantitative fashion (239). The most favorable experimental conditions to demonstrate these "heteronymous" responses are post-tetanic potentiation (40, 239), asphyxiation, and cooling of the cord, the latter having been shown to enhance the amplitude and duration of various spinal potentials (58, 220). The second problem of myotatic unit activity is the mode of transmission of impulses to motoneurones of antagonist muscles of the same unit. It has been shown by Eccles *et al.* (130, 131) that the muscle spindle afferents (group IA fibers) excite not only the motoneurones belonging to their synergic muscle group, but also interneurones in the intermediate nucleus. The tendon organ afferents (group IB) make no direct connections with motoneurones but are widely distributed to the intermediate nucleus interneurones at the segmental level. These interneurones would act as inhibitory "commutators" (165) on the antagonist motoneurones. Hence, contrary to previous opinions, antagonist inhibition would be disynaptic and not monosynaptic. In summary, the myotatic unit is organized on the principle of a double reciprocal innervation, the spindles facilitating their own synergic muscles and inhibiting their antagonists, the Golgi tendon organs doing the reverse. This organization however is not symmetrical (165).

Two other inhibitory mechanisms must also be considered: (*a*) the reflex inhibition attributable to interaction between dorsal root fibers which leads to a blockage of the afferent impulses before they reach the spinal cells (184), and (*b*) the inhibitory effects mediated by the recurrent axon collateral which stimulates (probably by a cholinergic mechanism) interneurones located in the ventral horn ("Renshaw cells") and whose axons in turn affect the motoneurones (128, 129).

Although after acute deafferentation of a limb $\alpha$ motoneurones are greatly depressed, chronic deafferentation is associated with a tonic adaptation and exaggeration of postural reflexes attributed to a sensitization of the partially denervated spinal neurones to nervous impulses reaching them from various receptive fields (341). As motoneurone hyperactivity may persist when all known sources of postural tone are destroyed this seems to demonstrate the existence of an automatic continuous central tonus (343). It has also been shown that exteroceptors may participate in the maintenance of postural tone (75) and that special muscles, like the sphincter ani, are controlled by the same reflex mechanisms as other skeletal muscles (48). Finally interesting results have been obtained in man by the school of Hoffmann (138, 315) and by Paillard (189, 275, 276, 277) on the silent period and the interplay of peripheral and central factors in myotatic reflexes and on clonus (186).

*Motor control over muscle spindles.*—Reference has already been made to the extensive discussion by Granit (165). Only a few new points can be stressed here. Kuffler was able to establish by stimulation of the motor fibers in lobster and crayfish the existence of a "fast" muscle bundle connected with the rapidly adapting sensory neurones and of a "slow" muscle bundle linked with the afferent fibers giving a well maintained discharge pattern (223). Decerebrate rigidity obtained with the classical Sherrington method (intercollicular section) and with the "anemic" method of Pollock & Davis is not identical. In the anemic preparation $\alpha$-$\gamma$ linkage is lacking, standing being maintained by pure $\alpha$ activity. These divergent results have disclosed the fact that the anterior part of the cerebellum (destroyed by the anemic method) plays an important rôle in organising the $\alpha$-$\gamma$ linkage (165). By iterative stimulation of the motor cortex it is possible to activate spindle afferents by way of $\gamma$ efferents, these latter being powerful enough to eliminate the arrest of discharges normally present during contraction (165, 166). Tonic facilitation of the ventral horn cells is maintained by the spindle loop; in many cases (skin reflexes, postural reflexes, reticular control) $\alpha$ and $\gamma$ reflexes are excited or inhibited together, but often $\gamma$ reflexes fire first and so muscular sensory organs are immediately ready to "measure" during the ensuing contraction (165).

*Flexor and other reflexes.*—Laporte has started a systematic study in cat and frog of the spinal coordination of the ipsilateral flexor responses elicited by stimulation of afferent nonmedullated fibers (C group) conveying nociceptive stimuli. No ipsilateral extension may be obtained from this fiber

group. The reflex discharge to C fiber stimulation, recorded from ventral roots, is more important and appears later than the response to $\delta$ group stimulation and lasts 0.6 to 0.7 sec. in the spinal cat. In the spinal frog, C volley responses summate with "flexogen" A volleys and inhibit ipsilateral extension elicited by "extensogen" A volleys; conversely A "extensogen" volleys inhibit ipsilateral flexion of C group origin (225, 226, 227). Studying visceromotor reflexes elicited by stimulation of the great splanchnic nerve, Downman has shown that the effects obtained depend on afferents in the smaller myelinated fibers; the central paths of this multisynaptic flexor arc are longer than those of reflexes evoked by stimulating intercostal nerves and a conspicuous central inhibitory influence is disclosed (125). Further studies include work on spinal shock (22), segmental innervation of the cat's hind limbs (204), reflexes in relation to sexual behavior integrated at the sacral level (72), and a reflex contraction of the abdominal muscles arising from the distension of the vagina of the fowl in oviposition (334).

*Muscular activity.*—Studies on motor unit activity have shown that there is a multiple innervation of a single muscle fiber (141, 191), have disclosed the significance of the double (myoseptal) innervation of the trunk muscles in the catfish (107), and have analysed the gradation of muscular contraction in man (42, 269).

## Brain Stem Reflex Activity

Besides the several functions attributed to the reticular formations, different mechanisms subserved by more specific brain stem structures and pathways have been investigated. In an extensive anatomical study, Scheibel & Scheibel have described the cells and dendritic patterns in the inferior olive as well as their topographical distribution and have drawn functional deductions about the olivary neuropil (313). Other anatomical contributions deal with the morphological differentiation of the avian inferior olive (352), the quantitative evaluation of the evolution of the cell size and number in the mesencephalic nucleus of the trigeminus through the stages of development and metamorphosis in the frog (221), and the analysis of the fibers and connections of the fasciculus longitudinalis medialis (308).

Oculomotor mechanisms have been investigated with the following results: afferent discharges from stretch receptors in the extraocular muscles induce sustained responses along the course of the trigeminal ascending nucleus (attributed to primary afferent neurones) and late sustained responses in different parts of the brain stem (99, 100, 101, 146). Brain stem, telencephalic, and cerebellar pathways for eye movements and especially for centering of the eyes have been explored (36, 193, 366); the centering of the eyes is an integral part of a general system integrating body movements, behavior, and EEG arousal (36). The widely accepted conception of the Perlia's nucleus as a "centre for convergence" is not supported by available evidence (360). Like eye movements, "acoustic prehension" is integrated by brain stem structures (63). The compensatory nystagmus observed

after bilateral labyrinthectomy is shown to be attributable to a progressive increase in sensibility of vestibular centers to normally subliminal labyrinthine afferents (139, 140). Through the combination of stimulation, localized destruction, and degeneration techniques, Hassler & Hess have demonstrated that rotation of the head, eyes, and trunk about the longitudinal axis of the body is coordinated by the nucleus interstialis which is connected with more oral components through the tractus interstitiothalamicus and the fibers along it. This system is controlled by vestibular afferents either directly or indirectly through the cerebellum, and its continuous tonic activity is mediated by the tractus interstitiospinalis (173).

The reflex discharges of the electrical organs of some fishes are coordinated at the brain stem level: in *Torpedo* the afferent path relays in a bulbar formation (labelled the nucleus ovalis) whose localization, histological constitution (335), and electrical activity (12) have been described. This formation controls the discharges of another well known bulbar structure, the electrical lobe, at which level the synaptic transmission has been studied with extra- and intracellular microelectrodes (8). In *Raja clavata* and *Electrophorus* a central formation controlling the electrical discharges has also been localized at the bulbar level (103, 336).

### Corticospinal Mechanisms

Lassek has given a succinct account of anatomical, physiological, and neurophysiological work up to 1951 on the pyramidal tract (228). The structural and functional maturation of the corticospinal tract has been studied in an especially well suited material, the pouch-young opossum (359). In the adult mammal no attempt at regeneration has been observed after a surgical section of the pyramidal tract: the axons degenerate first to a proximal node of Ranvier, then to their last collaterals (224). The cortical origin of the pyramidal tract mapped by antidromic volleys is more extensive than the traditional "motor cortex" and includes almost the whole of the first somatic sensory area (291). The cortical representation of the direct corticomotoneuronal system has a smaller extent than the cortical field from which late and tonic discharges can be evoked in peripheral nerves (38). In the human cortex representation areas of neck and trunk movements are contiguous (270).

Cortical mechanisms responsible for pyramidal activity have been studied by recording with microelectrodes the electrical discharges in the pyramidal tract. It has been pointed out, however, that at the decussation level these responses may be contaminated by the spike activity of extrapyramidal units (281). The pyramidal responses to single shocks applied on the pericruciate or the somatosensory areas consist of an early stable deflection attributable to the direct activation of pyramidal neurones, followed by a series of later deflections caused by the indirect or relayed excitation of pyramidal neurones through cortical interneurones (281). The variations in amplitude or in area of the pyramidal responses may be used

as an index of the variations in the threshold or sensitivity of the cortical or related interneurones (373). Through this latter method it has been demonstrated that the intracortical events responsible for arousal do not affect the responsiveness of the corticospinal neurones (56), while a positive polarisation of the cortex increases the pyramidal discharges which also becomes continuous (251).

At the level of the spinal motoneurones the earliest detectable sign of voluntary sensory-motor reaction is the antagonist inhibition (187). During the after discharge set up by a repetitive stimulation of the cortex, a facilitation of the monosynaptic reflex followed by an inhibition is observed (39). Although the cortical control of vegetative functions is beyond the scope of this review, we think it worth mentioning the fact that a regular concomitant of the corticospinal activation is an immediate rise in blood pressure mediated by collateral fibers leaving the pyramidal tract at the bulbar level to reach the vasomotor centers (260, 261). Only in the cases where the cortical activation turns into an epileptic activity is the early pressor effect followed by a much greater and more delayed hypertension closely related to the spread on the cortex of the convulsive discharges (258, 259). Motor effects of various types of cortical lesion and of lesions of the precentral gyrus have been compared (321).

## SOME INTRACORTICAL MECHANISMS

The possibility has been discussed of defining cortical areas by the frequency of cells (counted by a scanning densitometer) expressed as a function of depth (71), and it has been pointed out that the cortical neurones density and the activity of the acetylcholine (ACh) system bear similar relationships to brain size (347). During the development of the chick embryo, appearance of the strychnine spike response and changes of the spontaneous activity have been correlated with biochemical and cytological changes (23).

Studies of cortical events using both extra- and intracellular microelectrodes have shown more and more the importance of the dendritic potential. Presynaptic and postsynaptic axonal responses, cell body responses, and the much longer and slower dendritic responses can all be differentiated according to their latencies and their shapes in central pathways (340). In a pyramidal cell of Ammon's horn the electrical response shows an important late negative phase reflecting a membrane hyperpolarization, which is probably of dendritic origin (69). Studies on the cortical pyramidal cells, directed especially to the properties of the apical dendrites, have demonstrated their decremental and nonrefractory responses (88). The significance of these apical dendritic potentials has been discussed with reference to the field of potential around the neurone soma, repetitive discharges, brain waves (88, 340), and to a possible inhibitory action on cells located in deeper layers of the cortex (332). Conditions required for spontaneous firing in isolated cortical slabs have been related to the stimulating influence of circulatory and humoral factors (197) and also to the different rates of re-

covery of resting membrane potentials. It is thought that the deep somatic ends of the neurones conducting the burst responses repolarise more slowly than do the superficial ends (64), a hypothesis which has been tested on a model network (65).

The rôle of the chemical transmitters (and especially of acetylcholine) which are involved in the spontaneous (194) and evoked activity (83) of the cortex has been discussed in a general review by Feldberg (in 268).

Interesting information has been given by the application of new techniques for studying potential distribution. Lilly analysed the findings obtained with his 25 electrode recording system. In his resulting "figures," which occur in relation to spontaneous activity (237) or to responses to click in the acoustic cortex (236), he chooses as important characteristics the "leading" and "trailing" edges and describes their position, speed, and direction of travel. He draws suggestive conclusions on their relationships with intracortical events. Potential distribution analysis allows the differentiation between the electrical activity produced by stationary sources which are attributable to changing source configurations (322, 323). A new toposcopic technique discloses the travelling of certain EEG waves (285). Isolated cortical slabs are provided with the mechanisms necessary for the 3 c./sec. petit mal discharges (196, 371). Indication on cortical mechanisms has been drawn from studies on abnormal activities such as spreading depression (60, 61, 170, 171, 172, 212, 244, 302), epileptic discharges (87, 201, 358), strychnine foci (90, 331), and from the action of cortical hypoxia on conditioned reflexes (81, 245).

## NONSPECIFIC DIFFUSE SYSTEMS

Reference has already been made to the important symposium on *Brain Mechanisms and Consciousness* which has appeared in book form. Here the main information concerning the diffuse systems of the brain stem were presented by Magoun, Moruzzi, Bremer, and Jasper (47). Detailed anatomical data on the brain stem reticular formation has been given by Olszewski (47) and by Hugelin (188). Those concerning the diffuse thalamic system may be found in the contribution of Nauta & Whitlock to this symposium (47). Two atlases of the diencephalon with the Horsley-Clarke coordinates, one for the cat (202) and the other for the rabbit (310) are available.

### Neural and Humoral Control of the Brain Stem Reticular Formation

*Neural control.*—The complete topography of reticular responses to photic stimulation (including latencies) has been mapped out over all central brain stem structures (198). Evidence has been given that the vestibular system influences the reciprocal action of the reticular formation (RF) on the spinal motoneurones (159), and there is some indication that trigeminal and olfactory impulses are transmitted to the inhibitory bulbar formation (15). Corticoreticular projections have been demonstrated by different

methods (147, 320, 348), and it has been suggested that cerebelloreticular connections are responsible for the autonomic manifestations elicited by cerebellar stimulation (374). A high voltage, brief duration somatic response has been recorded and analysed in the medial RF, at the tectothalamic junction (93). A schema of the possible pathways for the different modalities of afferent impulses to reticular cells has been tentatively proposed by von Baumgarten & Mollica (30).

Evidence for the control of reticular activity by interoceptors (chemoreceptors of the carotid body) has been obtained by the analysis of the EEG arousal observed in the cat at certain stages of hypoxemia (44, 117). This reflex activation probably explains the anoxic stimulation of the hypothalamic electrical activity observed by Porter (290) and the intensification of the $\alpha$ waves of the human EEG reported by Jung in a general article on the effects of hypoxia (208). The marked inhibition of the spinal reflexes brought about by a rise of arterial pressure in the prepontine cat has been interpreted as being the consequence of direct stimulation of the inhibitory RF through bulbar projections of the vascular baroreceptors (119). This reflex mechanism probably explains the inhibition of the linguomandibular reflex observed during experimental hypertension in the isolated carotid sinus (287) and clinical observations showing relationships between alterations in EEG and novocainization of the carotid sinus region (279).

Microelectrode studies of reticular activity have thrown some light on the ultimate nature of the action of afferent impulses on the reticular elements. The important contribution of the Pisa laboratory to this problem has been reviewed by Moruzzi (47). In the same laboratory it has been established that the action of the corticoreticular afferents on the reticular cell is that of modulating the frequency of its discharge. When a unit is fired by cerebellar, sensory or cortical impulses, or both, the different "drivings" summate algebraically (31). The existence of true neuronal convergence of heterogenous messages has been confirmed (14, 30, 31, 174, 252), and blocking effects as well as facilitation between two responses of the reticular cell have been demonstrated and analysed (14, 174). The sharing of reticular elements by afferent impulses, though extensive, is, however, only partial: the same unit which is fired by one or several kinds of afferents may be unresponsive to other kinds (14, 174, 252, 314, 355). Even for sensory impulses from the same sensory modality (cutaneous), differences of latencies and of patterns of firing in relation to the peripheral origin of the message have been observed. It is suggested that space at the periphery is represented in the RF by temporal coding of impulses (355). Some indication of a spatial differentiation and organization in the reticular mass is given by the observation that patterns of responses are more tonic at the pontine midbrain level than at more anterior levels (14) and also by the fact that private connections and selectivity of responses seem to exist in the corticoreticular relationships (314).

*Humoral control.*—A large amount of evidence is accumulating showing

that humoral and biochemical agents are as important in the control of reticular activity as are messages mediated via nervous efferents.

(*a*) A powerful stimulating action of the blood $pCO_2$ on the ascending activating system has been observed (117), and experimental evidence has been given that an adequate $pCO_2$ is as necessary for the normal activity of the mesodiencephalic RF as it is for the respiratory center (44). Koella & Gellhorn have localized in the hypothalamic part of the ascending system the area stimulated by an excess of $CO_2$ (219). The same system is more sensitive to a reduction of the blood $pO_2$ than the other diffuse encephalic systems which control cortical activity (44). Small variations of blood temperature modify its excitability (218) and induce central effects which may be partly ascribed to a reticular action (151, 217).

(*b*) Numerous publications demonstrate that the two best known neurohumoral transmitters are intimately involved in the activity of the reticular cell. Literature concerning the central actions of acetylcholine has been reviewed by Minz (250) and by Feldberg (268). The previous observation of Bonnet & Bremer of the awakening action on the EEG of intracarotid injections of ACh has been confirmed by Longo in the conscious rabbit. This effect is observed for subpharmacological doses, is accompanied by motor responses, is duplicated by other cholinergic substances, and is reduced after atropinization (241). The demonstration of the meso-diencephalic site of both alerting effects of ACh and di-isopropylfluorophosphate and of blocking effects of atropine has been achieved (136, 298, 299). The action of the same drugs injected by the intraventricular route seems to be more drastic and less physiologic than those observed after intravascular injections. Results obtained by this method are suggestive but often contradictory (94, 144, 145). Concerning the rôle of epinephrine the conclusion that adrenergic neurones are present in the reticular network has been drawn (119) from the following facts: (*a*) It is possible to extract from the brain, in formations the extent of which is approximately the same as that occupied by the RF, significant amounts of "sympathin" probably liberated *in situ* (351). (*b*) Circulating epinephrine stimulates directly the ascending (43) as well as the descending reticular systems (119). The existence of an adrenergic link in the reticular chain would explain: (*i*) the stimulating effects of epinephrine on the waking state (199) and on the spontaneous motor activity (179) of conscious animals, as well as epinephrine's specific action on the activity of the supramamillary region (290), (*ii*) the central excitatory effects of the amphetamines (structurally related to the sympathetic amines) the direct action of which on the ascending reticular system has been demonstrated (136, 180), and (*iii*) the central depressive actions of antiepinephrine compounds such as chlorpromazine (26, 29, 108 to 111, 181, 242, 342). (*c*) Various observations, too numerous to be quoted here, suggest that endocrine factors may influence reticular activity directly or indirectly. The most direct demonstration is that of Porter's who, using stereotaxic recording, has shown that ACTH decreases the spontaneous electrical discharges

of the anterior brain stem and completely abolishes the stimulating action of epinephrine on the same region, and also that the normal responses are restored by the administration of cortisone (290).

## Caudal and Cephalic Influences of the Brain Stem Reticular Formations

To the universality of the messages reaching the brain stem reticulum corresponds the universality of the reticular influences which, controlling simultaneously the level of activity of sensory, motor, cortical, and autonomic functions, contributes largely to the higher processes of consciousness and global behavior.

It has been confirmed that the RF is able to control the sensory input. Reticular stimulation may provoke alterations in muscle spindle activity (134, 165), long lasting facilitory effects on the response to flash of retinal neural units (165), and depression of the postsynaptic afferent impulses in the gracilis (317) and trigeminal (176) nuclei. Such reticular control may perhaps contribute to the depression (256) or facilitation (158) of evoked cortical potentials observed during reticular stimulation, and it has been suggested that selective reticular inhibitory effects may account for the "habituation" to external stimuli (175).

At the spinal level the stimulation of the suprabulbar RF provokes a diphasic facilitation of both flexor and extensor monosynaptic reflexes and of the antidromic potentials; it also decreases the latencies of the reflexes. The latter phase of this facilitation may summate with posttetanic potentiation (215). In some cases an inhibition of the monosynaptic reflex is obtained from points localized in the same suprabulbar region, but this effect is never accompanied by an inhibition of antidromic firing or of posttetanic potentiation which are actually facilitated (57). Undeniable reciprocal effects on antagonist muscles have been evoked from the bulbar RF (160). Inhibitory and facilitory actions of reticular stimulation on spinal autonomic reflexes have been reported (357).

Dynamic interactions occur between the antagonist systems superimposed or intermingled in the brain stem. The analysis of this hierarchy of influences has been in part achieved by means of successive transections of the brain stem isolating the different levels of the RF. Taken as a whole, the results show that the "downwards" facilitating action of the anterior part of the RF is normally checked by telencephalic influences (106, 119). When released by a diencephalic section, this facilitory action has prevalence over bulbar inhibitory effects (106, 119, 357), and a lower prebulbar section is necessary to unmask the inhibitory bulbar influence (119, 195, 357). A tonic inhibitory control of the bulbar inhibitory formation by pontine or suprapontine structures has been directly postulated (106, 195). From recent general articles on the respiratory "center," which is after all neurones "loosely scattered through large portions of the RF," it is clear that the same type of dynamic interactions play an important part in the central

command of the respiratory motoneurones [Wyss (372); Hoff & Breckenridge (183); and in (149)]. There is some indication of a differential sensitivity to physical factors among the different brain stem systems (28).

The main neurophysiological basis of the function of the ascending activating system may be found in the articles written by Magoun (47) and by Bremer (49). The closely related question of the regulation of the sleep-waking state has been discussed in two general papers (in 47, 118). Further precision has been obtained on different aspects of this ascending control. The critical decerebration level for obtaining a preparation having the "sleeping" behavior of the "cerveau isolé" lies immediately anterior to the roots and sensitive nucleus of the trigeminal nerve (303). The sleeping condition induced by chronic elimination of the posterior hypothalamus is only transitory but may reoccur after lesions in the dorsal thalamus (330). The anterior part of the reticulate nucleus, with the posterior part of the internal capsule, form the main route which conveys the reticular impulses to the cortex (216), and it has been inferred that the reticular ascending system represents the common multisynaptic relay station for "secondary" cortical responses (295). An interesting demonstration of an extraneuronal control of the cortex by the activating system has been given (197). Of much interest are Caspers' studies: stimulating brain structures in the conscious rat, he has been able to induce from the massa intermedia and the mesencephalic central gray either the whole range of the frequency and form of the sleep electroencephalogram or the whole range of the vigil electroencephalograms, according to the simuli intensity. Both effects may summate with arousing or depressing actions of sensory stimuli and are accompanied by appropriate behavioral changes (80). A constant parallelism is regularly observed between the fluctuations of cortical or behavioral activity and vegetative tonus (78, 79, 80). The concomittant gastrointestinal and emotional disturbances induced in the monkey by chronic hypothalamic stimulation is another example of somatovegetative integration (148).

The psychophysiological implications of all this data have been expressed and discussed by Gellhorn (157), Pond (289), and several participants of the Ste. Marguerite symposium [Penfield, Gastaut, Grey Walter, Hebb, Lashley, Kubie (47)]. In a highly thoughtful and philosophical paper Fessard has integrated all our present knowledge of the nervous activity which may guide us to an understanding of the neural basis of consciousness (47).

### The Nonspecific Diffuse Thalamic System

Jasper has summarised his views on the organization of this system from the results obtained in his laboratory up to 1953 (in 47). In a more recent study, stimulating the different thalamic diffuse projection nuclei with just supraliminal intensity, Jasper *et al.* have cleared up the topographical organization of these cortical projections and confirmed the possibility that recruiting responses may be evoked in sensory cortical areas (203). A study

of the relationships between cortical recruiting waves and cortical unit activity has shown that, during the periods of spindle bursts, separated trains of spikes related in time and phase with the individual wave of a spindle, appear on the microelectrode record, whereas only random spike discharges are observed during cortical arousal. It is postulated that the positive phase of the slow wave is built up by the summation of the positive afterpotentials of the corresponding train of spikes (350). A marked inhibition of the movements evoked from the motor cortex is regularly observed during the recruiting waves induced by stimulation of the anterior thalamic nuclei. These results are regarded as indicating that the recruiting potentials represent the cortical equivalent of descending inhibition (167).

## CEREBELLUM

An important synthesis of the cerebellar anatomy (morphology, structure, connections) edited by Jansen & Brodal (200) contains a most interesting chapter on "The Structural Organisation of the Cerebellum" written by these two authors. The physiopathology of the cerebellum has been discussed by Miller-Guerra (249).

*Afferent side.*—Cerebellar projections from the paramedian reticular nucleus (situated close to the bulbar inhibitory formation) (54) and from the peri-hypoglossal nuclei (functionally related to motor activity of the tongue) (344) have been traced by the retrograde degeneration method. Visceral impulses, possibly of nociceptive type, mediated by the $\delta$ fibers but not by the coarse afferent fibers of the splanchnic nerve, reach the cerebellum, in company with impulses from high threshold somatic afferents (367). The distribution and temporal patterns of somesthesic afferent impulses firing the paramedian lobule have been studied (337). These impulses interact with afferent volleys from the somesthesic cortical areas, but it does not seem that the interaction takes place in the same cortical cerebellar final element (11).

*Intrinsic activity.*—The slow activity (less than 60 c./sec.) superimposed on the classical 300 c./sec. electrocerebellogram of the cat seems to be attributable in greater part to cerebral influences and to a lesser degree to peripheral afferent volleys (95). Microelectrode analysis of the complex potential found on the cerebellar cortex following photic stimulation demonstrates that the slow and delayed component of this potential is a result of the "reflex" response of the effector Purkinje cells (68). An anatomical study of the synaptic relations of the afferent climbing fibers suggest that afferent impulses may circulate in adjacent neuronal chains of varying length and complexity including the dendrites of Purkinje cells (312). In the kitten the progressive increase in the excitability in the anterior lobe has been correlated with the progressive differentiation of the Purkinje and basket cells (86).

*Efferent side.*—New cerebellar efferent connections with the medial and dorsal longitudinal fasciculi have been found (297). In two important papers,

Carrea & Mettler described the origin, constituents, and distribution of the three limbs of the brachium conjunctivum in *Maccacus rhesus* (76). They found that tremor occurs with the interruption of pathways which project rostrad towards the thalamus and the globus pallidus but not towards the subthalamic nuclei (77). Cerebellar ataxia is produced by total abolition of the outflow through the brachium conjunctivum (247). Moruzzi has reviewed the question of the reflex and automatic components of postural tone and its control by the anterior lobe of the cerebellum (262). Recent contributions of the Italian school to this problem deal chiefly with the conditions in which stimulation or destruction of cortical or nuclear cerebellar structures leads to facilitation of postural extensor tone (84, 85, 263, 288). Evidence of cerebellar influence on the cerebrum has been obtained (73, 124, 327). The existence of a cerebellar control of ascending sensory impulses has been postulated (30). Discrete and well coordinated motor responses have been evoked from the cerebellar surface, even after destruction of the motor cortex (115).

## THE LIMBIC SYSTEM

Under this denomination are grouped heterogeneous morphological units which fail to meet the criteria defining typical isocortex, allocortex, and juxtallocortex, together with closely related subcortical structures (294). In general accounts Fulton (149, 150) and MacLean (243) insisted on the primacy of the rôle of the limbic system in emotional behavior and called it a "visceral brain," this term being used with its sixteenth century meaning. Pribram & Kruger having reviewed critically all the available data propose a new classification of these limbic structures (294). Anatomical studies have examined in a quantitative way the mammillary bodies and their connections (169). The precise topographical organisation of the mammillothalamocingular system (104, 292), the termination of the efferent fimbria fibers (293), and the connections of the stria medullaris (62) and of the claustrum (296) have been described.

On the afferent side it has been found that during the stimulation of sensory pathways or of the brain stem reticular formation an undulating series of large slow waves appear in the hippocampal records (168, 280). This specialized "paleocortical arousal" is induced by projections from the septum running in the dorsal fornix and is conducted to the hypothalamus and the thalamus through the fornix proper. The cortical slow waves are synchronous with bursts of spike activity which can be recorded from single pyramidal cells in Ammon's horn (20, 21). On the other hand, cortical arousal may be obtained by stimulation of the amygdala. During arousal the amygdala itself presents a fast rhythmical activity occurring in spindles (142).

On the efferent side, Gloor, using the evoked potential method, has demonstrated widespread projections from the amygdaloid nuclei to cortical and subcortical structures (including the extrapyramidal motor system and different parts of the hypothalamus, subthalamus, and brain stem reticular

substance) (161, 162). Numerous relatively direct projections from the tip and the superior gyrus of the temporal lobe to subcortical and rhinencephalic structures have been found with the EEG technique (319). These projections may well explain the multiple motor and vegetative effects obtained by stimulation of the amygdala. In the animal deprived of its neocortex, section of the fornices results in a lowering of the rage threshold (305). After amygdalectomy young rhesus monkeys though appearing more aggressive in the individual-cage situation are, however, less dominant in the group-cage situation (304). In the cat, bilateral ablation of the cingulate area is followed by confused perseveration, obsessive behavior, and a plastic tendency to remain in bizarre positions (213). The hypomotility produced by bilateral temporal pole ablation disappears after fronto-orbital lesions although inattention and apathy remain (214). Amygdalectomized monkeys (but not animals with lesions of the hippocampus or cingulate) require more training than the intact animal in the acquisition of a conditioned avoidance response but show no decrement in the retention of the response acquired before the operation (46). To induce hyperactivity in the rat, lesions of the anterior brain must involve rhinencephalic structures (328).

Regarding sexual functions, limited lesions in the basal part of the preoptic area provoke in female as well as in male rats a typical copulatory behavior which seems to be highly independent of the sex hormone activity (182). Enhancement of the electrical activity in the rhinencephalon (experimentally induced by intraventricular histamine) is an essential step in the process bringing about the release of ovulating hormone from the adenohypophysis (309). After rhinencephalic lesions the subsequent rage outbursts are accompanied by a discharge of the pituitary-adrenocortico-thyroid system (137). Other papers deal with hippocampal (234, 278, 286) and temporal (143, 185) seizures and neurosurgical observations (24, 82, 230, 318).

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# VISCERAL FUNCTIONS OF THE NERVOUS SYSTEM[1,2]

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The visceral functions of the nervous system are so intimately related to the somatic and higher functions of the nervous system that the selection of papers for an annual review is quite difficult. For example, acetylcholine acts at central, somatic, and visceral neuroeffector junctions and on structures having no nerves. Furthermore, acetylcholine affects the heart and peripheral circulation and is involved in the functioning of visceral receptors. Where, then, shall the studies on the action, formation, and destruction of this ubiquitous agent be considered? Some are reviewed here. A new drug is introduced to control hypertension but soon ends up being used primarily in psychiatry. Does this drug affect the visceral functions of the nervous system? Should papers concerning its central effects be reviewed here? The answer in this case has been, in general, no. The following, therefore, is a short review of some of the literature on some of the visceral functions of the nervous system.

*Posterior pituitary.*—Sir Henry Dale (1), in the Banting Memorial Lecture, 1954, informally reviewed six decades of study of the posterior lobe of the pituitary. In describing the neural control of hormone production Dale draws an analogy between the hypothalamicohypophyseal system and the better defined neurohumoral transmitter functions of the motor nervous system. He cautions, however, against regarding this analogy as completely true.

Ethanol, in the form of 120 ml. whiskey administered orally, increased urine output and free water clearance, and decreased the sodium, potassium, and chloride excretion in normal males [Rubini, Kleeman & Lamdin (2)]. The authors believe these results substantiate the idea that ethanol inhibits the supraopticohypophyseal system. If the ethanol was administered at the height of water diuresis, no effect on urine volume or solute excretion was observed. Ethanol also prevented or diminished the antidiuretic effect of hypertonic saline or venous congestion in water-loaded subjects. Kleeman *et al.* (3) state that ethanol administered prior to an antidiuretic-hormone-provoking stimulus effectively blocks the stimulation of the release of ADH. Pearce & Newman (4) found that ethanol inhibits orthostatic antidiuresis but does not inhibit orthostatic inhibition of salt excretion. By wrapping the legs with elastic bandages prior to standing, inhibition of salt excretion was prevented but the antidiuresis was variably affected. These authors believe

[1] The survey of literature pertaining to this review was concluded in June, 1955.

[2] The following abbreviations have been used in this chapter: ADH (antidiuretic hormone); ACTH (adrenocorticotropic hormone); TEA (Tetraethylammonium); ATP (adenosinetriphosphate); ADP (adenosinediphosphate); DFP (di-isopropylfluorophosphate); ECG (electrocardiogram).

that two separate mechanisms control postural adjustments of water and salt excretion, water being under posterior pituitary control, salt being under control of some mechanism sensitive to distribution of interstitial fluid.

In the rat, Fingl *et al.* (5) showed that a dose of ethanol sufficient to prevent osmotic activation of the supraopticohypophyseal system does not prevent release of ADH by nicotine or morphine. Winter, Gaffney & Flataker (6) found that nalorphine (Nalline; *N*-allylnormorphine) effectively blocks morphine-induced antidiuresis in rats, and also found that nalorphine by itself is not an antidiuretic. Their evidence suggests that morphine, acting on the hypothalamus, stimulates release of ADH. Crawford & Pinkham (7), on the other hand, believe that morphine does not stimulate ADH release. It is their opinion that morphine has two antidiuretic actions: (*a*) a change in renal hemodynamics, and (*b*) a potentiation of preformed ADH.

Dempster & Joekes (8) using faradic stimulation of the flanks of dogs to produce "emotional" antidiuresis have studied the two separable phases of this phenomenon. The immediate, profound antidiuresis of short duration is apparently mediated by adrenal medullary secretion (epinephrine). The secondary, prolonged antidiuresis of slower onset is mediated by ADH.

Work on a cycloergometer increased the antidiuretic activity of the venous blood of normal human subjects [Vera & Croxatto (9)]. The antidiuretic activity of the blood as determined by using the hyperhydrated rat bioassay was not increased if 650 ml. of water were ingested immediately before work.

The interesting studies of Abrahams & Pickford, reviewed last year from abstract by Burn (10), on the relationship of pituitary secretion to urine flow and uterine motility have been published in full (11). Landgrebe & Mitchell (12) described a method for making a strongly active melanophore-dispersing extract of the intermediate lobe of the pituitary.

*Hypothalamus.*—The effects of changes in body temperature on the cardiovascular responses to direct hypothalamic stimulation in the cat have been studied in some detail by Gellhorn, Koella & Ballin (13, 14). Temperature changes were achieved by placing the anesthetized cat in a large water tank. At 25° to 29°C. the changes in response to hypothalamic stimulation, and acetylcholine and epinephrine injections suggested a decreased excitability of the sympathetic centers. At 30° to 33°C. there was an increased reactivity of parasympathetic and sympathetic centers. At 40°C. there was also an increased excitability of the sympathetic centers.

The hypothalamic pressor response in cats can be diminished by reserpine as shown by Harrison & Goth (15), or abolished by chlorpromazine as described by Dasgupta & Werner (16). Reserpine also decreases the responsivity of the medullary vasopressor center [Bhargava & Borison (17)].

French *et al.* (18) tested the effect of daily stimulation of the hypothalamus on the gastrointestinal tract of monkeys. Six out of ten test animals developed gastric or pyloric lesions after at least 30 days. During the periods of stimulation these six animals showed gastric hyperacidity and developed occult blood in the stools. The lesions are thought to be attributable to

changes in motility and blood supply mediated by the autonomic nerves. The authors compare these results with peptic ulceration in man.

By means of destructive electrolytic lesions, Anand, Dua & Shoenberg (19) have localized, in cats and monkeys, a "feeding center" in the lateral hypothalamus, and a "satiety center" in the medial hypothalamus.

*Sympathetic nervous system.*—The participation of the venous system in pressor reflexes, and the venomotor tone during hemorrhage and shock have been examined in some detail by Alexander (20, 21). In brief, his method consists of measuring venous distensibility in a blood-perfused, isolated, but innervated intestinal loop. Venous constriction occurs during central vagal stimulation, carotid sinus hypotension, hypercapnia, hypoxia, and hemorrhage. Venous dilation occurs with carotid sinus hypertension and during irreversible hemorrhagic shock. The method will be useful in examining many vascular phenomenon which cannot easily be explained by changes on the arterial side alone.

Stimulation of the sympathetic efferent nerves to the carotid sinus decreases the arterial blood pressure and reduces the carotid sinus hypertensive reflex [Kezdi (22]. The local application to the carotid sinus of the adrenergic blocking agent phentolamine (Regitine) prevents this effect. It has been shown that the baroceptor activity of the carotid sinus can be changed by the contractile state of the wall of the sinus. Kezdi, therefore, postulates that disturbances of the sympathetic innervation to the carotid sinus might be part of the pathogenesis of hypertension. Wakerlin *et al.* (23) constricted the carotid sinus area in dogs by means of a special plastic clamp. This resulted in a significant, sustained hypertension.

Uvnäs (24) has reviewed the sympathetic vasodilator outflow. He and his students have done much to describe this system. Their method of approach is well illustrated by the description of the role of the hypothalamus as a relay station [Eliasson, Lindgren & Uvnäs (25)]. This dilator outflow is cholinergic, is probably confined to skeletal muscle, and originates in the motor cortex. It is postulated that the sympathetic dilator system acts to increase blood flow in skeletal muscles in situations requiring sudden muscular effort.

Stimulation of the cut central end of the lumbar sympathetic chain between the first and third lumbar ganglia can produce vasoconstriction in the opposite hand. Cooper & Kerslake (26) found this in three out of nine experiments in humans under nitrous oxide anesthesia. One suggestion put forth by the authors as to the origin of the afferent fibers stimulated is that these may be afferents from the leg concerned in the reflex vasodilator response to heating of the skin.

Douglas (27) has demonstrated quite conclusively that the vasoconstriction produced in the rabbit ear by bacterial pyrogen is brought about entirely by sympathetic vasoconstrictor nerves. Removal of the stellate ganglion or administration of the ganglionic blocking agent, hexamethonium, completely abolishes this vasoconstriction.

Kao & Ray (28) by means of a complex cross-perfusion technique have separated the neurogenic and humoral controls of cardiac output during exercise. The neurogenic increase in cardiac output is accompanied by a decrease in the arteriovenous oxygen difference, the humoral increase by an increase in arteriovenous oxygen difference.

The relationship of the adrenal medulla to the mechanism of action of analgesic agents has long been the subject of study. McCoubrey (29) tested the ability of four α-phenylethylamines, one of which is an analgesic, to deplete the adrenal of ascorbic acid in rats. All were effective. The analgesic member was the most effective in depleting adrenal cholesterol. None of the amines caused the release of epinephrine. Miller *et al.* (30) could find no evidence that morphine analgesia is mediated by epinephrine release from adrenal medulla. On the contrary, their evidence indicates that there is no relationship between the analgesic action of morphine and its ability to induce epinephrine release.

The actions of histamine and pilocarpine on the superior cervical ganglion and the adrenal medulla have been studied by Trendelenburg (31). Both agents act as stimulants to the ganglion or gland and are not blocked by hexamethonium. Atropine specifically blocks pilocarpine while histamine can be blocked by an antihistaminic agent. Wool *et al.* (32) have obtained evidence that the adrenal medulla and peripheral sympathetic nerves are necessary for mobilization of fat.

Three general methods for studying the adrenergic neuroeffector system have evolved over the years: (*a*) extraction, identification, and quantification of active material from adrenal medullary tissue and adrenergic nerves; (*b*) identification and quantification of circulating adrenergic neurohormones; and (*c*) comparison of the effector responses to epinephrine and levarterenol.[3]

*Adrenal medullary hormones.*—Human adrenals removed surgically from patients with carcinoma or hypertension contain about 0.5 mg. catecholamines per gm. of tissue according to Euler, Franksson & Hellstrom (33). About 84 per cent is epinephrine, the rest norepinephrine. In twelve cases of pheochromocytoma, Manger *et al.* (34) found that the tumors contained from 0.06 to 5.87 mg. of "epinephrine-like" material per gm. of tissue. In all but one of these the amount of norepinephrine was greater than that of epinephrine.

The structure of the catecholamine-containing granules in adrenal medullary cells has been further studied by Hillarp & Nilson (35). The granules were isolated from cow adrenal and subjected to various treatments including changes in tonicity, pH, and surface tension of the suspending media. Release of catecholamine was accompanied by protein liberation and a reduction in light-scattering by the granules. The evidence indicates that these

[3] The official name levarterenol is used in this review for the synthetic, levorotatory amine. For historical reasons, and to indicate its natural origin, the term norepinephrine will be used for the substance identified in tissues, secretions or extracts. It is presumed that the substances are identical.

granules behave as osmotic systems, have membranes impermeable to stored epinephrine and norepinephrine and contain a soluble protein fraction, and that the stored catecholamines are in a soluble or readily dissociable form.

Bander (36) has described two types of chromaffin cells in adrenal medulla. The "pikrinophile" or P-cells form, or contain, only epinephrine, and are found in the rabbit, guinea pig, and rat. The "fuchsinophile" or F-cells contain only norepinephrine and are found along with P-cells in the mouse, cat, and dog. These findings agree closely with previously published analyses of the epinephrine and norepinephrine content of adrenals from the various species.

Lockett (37) has identified a new catecholamine in saline extracts of the adrenals of cats, monkeys, and man; it appears to be isoproterenol (isopropylarterenol). Although she found this amine only in trace quantities, it is interesting to note that Lands & Tainter (38) have previously suggested the possible occurrence of this substance *in vivo*.

*Circulating adrenergic hormones.*—In a small group of normal human subjects Manger *et al.* (34) found 0.15 μg. of epinephrine per l. of plasma, and 3.96 μg. of norepinephrine. Euler, Franksson & Hellstrom (39) reported that after bilateral adrenalectomy in man the urinary epinephrine markedly declined while the norepinephrine content was frequently increased. This is suggested as evidence that the epinephrine excreted in urine is derived from the adrenals while norepinephrine comes presumably from adrenergic nerves.

Holzbauer & Vogt (40) could find no norepinephrine in the plasma of one human subject and several dogs. They also found that during insulin hypoglycemia the plasma concentration of epinephrine was significantly increased, but there was still no detectable norepinephrine. In cats Duner (41) showed that insulin produces a greater output of epinephrine than norepinephrine from the adrenals.

In venous effluents from liver, spleen, and intestine of cats and rabbits, Mirken & Bonnycastle (42) found primarily norepinephrine after electrical postganglionic sympathetic stimulation. In adrenal effluents, after splanchnic nerve stimulation, epinephrine was found to predominate. In general, their results are in accord with other studies. However, the quantities of catecholamines found, by chromatographic separation and bioassay, are somewhat higher than others have reported.

Nicotine administered intravenously to dogs elevates the blood epinephrine level. Using an isolated rat uterus bioassay for epinephrine, Kiser, Booher & Watts (43) found 50 μg. of epinephrine per l. after nicotine (0.1 mg. per kg.).

*Epinephrine versus levarterenol.*—In a short review Vogt (44) states, in comparing epinephrine and levarterenol, "thus noradrenaline is the more active compound on the cardiovascular system. . . ." Considering all of the comparative studies of epinephrine and its primary amine, not only on the cardiovascular system but also on other structures, this view is somewhat erroneous.

Epinephrine is more active than levarterenol in overriding the effects of acetylcholine on the electrogram of the isolated frog heart [Baker & Baker (45)]. McDougal & West (46) found epinephrine to be four times more active than levarterenol and 200 times more active than isoproterenol in inhibiting the peristaltic reflex in guinea pig ileum.

Hilton (47) found that the major difference between epinephrine and levarterenol on the isolated rabbit heart was the duration of the inotropic effect. Epinephrine showed the longer duration of action when identical doses of the two amines were used.

Celander (48) in an extensive study in cats found epinephrine to be more active than levarterenol in producing cutaneous and renal vasoconstriction, and in causing contraction of the spleen, iris, and nictitating membrane. In the skeletal muscle vascular bed epinephrine caused both dilation and constriction, while levarterenol produced only constriction. Celander considers the muscle vasodilation to be a metabolic effect of epinephrine similar to this substance's action in the liver. It is also his opinion that there is a clear cut functional difference between the two components of the sympathicoadrenal system; the neural component has predominance on innervated structure, while the adrenal medulla controls the noninnervated metabolic effects. Celander's suggestion, that the skeletal muscle vasodilating action of epinephrine is a metabolic effect, is open to debate. Variations in chemical structure of compounds closely related to epinephrine produce agents of varying activity. Isoproterenol, for example, which is an active vasodilator, has only slight metabolic activity as judged by its ability to produce hepatic glycogenolysis. In other words, there does not seem to be any correlation between metabolic activity and vasodilation.

Many blood flow studies in dogs have compared epinephrine and levarterenol. Spencer, Denison & Green (49) found epinephrine to be the more active renal vasoconstrictor when the amines were injected into the renal artery. Neither amine had any renal vasodilator action before or after effective doses of adrenergic blocking agents. When injected intravenously epinephrine is also the more active (50). Epinephrine is also more active than levarterenol in the dogs in producing femoral vasoconstriction, relaxing the intact ileum, or contracting the spleen. Only in producing a greater rise in arterial pressure and a more pronounced bradycardia does levarterenol appear more active than epinephrine (50).

Green *et al.* (51) showed that epinephrine and levarterenol were about equal in producing femoral vasoconstriction when injected intraarterially. When administered during a continuous isoproterenol infusion epinephrine became the more active constrictor and lost its secondary dilator effect. Youmans, Green & Denison (52) have further described the response of the vascular bed of canine skeletal muscle to epinephrine, levarterenol, and sympathetic nerve stimulation. Their results indicate that three types of "receptors" are present: (*a*) a vasoconstrictor mechanism, "alpha receptor," which is strongly activated by epinephrine, levarterenol, and nerve stimula-

tion; (*b*) a vasodilator mechanism, "beta receptor"; and (*c*) another vasodilator mechanism, "gamma receptor," which is activated by nerve stimulation, by methacholine, and perhaps by levarterenol, and which is blocked by atropine. Similar results are reported in another paper from the same laboratory [Green *et al.* (53)]. In studies on the mesenteric vascular bed Green *et al.* (54) found that its responses to epinephrine, levarterenol, and other amines were similar, though less intense, to those of the skeletal muscle bed. In the canine cutaneous vascular bed, the constrictor response was of the same magnitude but of greater duration than the response to levarterenol [Green *et al.* (55)].

Epinephrine and levarterenol briefly enhance, then depress, the vulnerability of the dog ventricle. Hoffman *et al.* (56) found them to be qualitatively and quantitatively similar in this respect. Collier, Meyers & Schmitt (57), using the dye dilution method for estimating cardiac output in normal or cardiogenic shock dogs, found that epinephrine increased cardiac output while levarterenol had no consistent effect. Cotten & Pincus (58) examined the comparative effects of epinephrine and levarterenol on the contractile force of the dog heart. In general, they found that the amines were qualitatively and quantitatively similar over a wide range of doses.

In man the cardiovascular responses to epinephrine or levarterenol infusion appear different. Dittmar, Mechelke & Nusser (59) determined these differences in six humans before and after the administration of the adrenergic blocking agent phentolamine (Regitine). Epinephrine, 30 μg. per min., before phentolamine increased systolic pressure, pulse pressure, and heart rate; the diastolic pressure was slightly decreased. After phentolamine, 20 mg. intravenously, both systolic and diastolic pressures were decreased by epinephrine, and the increase in heart rate was greater than before the adrenergic blockade. Levarterenol, 30 μg. per min., in the control state increased both systolic and diastolic pressures wih no change in pulse pressure; the heart rate was decreased. After phentolamine, levarterenol decreased both systolic and diastolic pressure and increased pulse pressure and heart rate.

In general, the results of all comparative studies of epinephrine and levarterenol, in any species, are consistent with the dualistic receptive mechanism theory which has recently been more extensively described (60). Furchgott (61) has described the use of isolated strips of rabbit aorta to differentiate these receptors. Further evidence for classifying the receptor for adrenergic relaxation of the intestine with the receptors associated with vasoconstriction has been found by Rothlin, Konzett & Cerletti (62).

*Amine oxidase.*—The relationship of this enzyme to the normal action of epinephrine has become more obscure. Burn, Philpot & Trendelenburg (63) showed that the amount of amine oxidase in the nictitating membrane declined after denervation. They concluded that this explained the increased sensitivity of this structure to epinephrine. Later, however, Burn *et al.* (64) found that iproniazid (Marsilid, isonicotinylisopropylhydrazine), a specific

amine oxidase inhibitor, did not sensitize the nictitating membrane to epineprine. Furchgott *et al.* (65) found that iproniazid did not sensitize rabbit aortic strips to epinephrine or levarterenol but did increase the sensitivity to tyramine.[4] It is now the opinion of Burn (63) that it is alterations in pseudocholinesterase activity, brought about by denervation or drugs, that change the sensitivity of effector cells to epinephrine and related amines.

*Amines in tissues.*—Duner, Euler & Pernow (66) determined the catecholamine content of various portions of the mammalian eye. No catecholamines were found in the lens, vitreous body or retina, and only small amounts in the aqueous humor. The ciliary body, lens, and choroid contained between 0.3 and 0.4 μg. norepinephrine per gm. of tissue. Much less epinephrine was found. Östlund (67) has described methods for determining catecholamines, and has measured the amounts present in various species including earthworms, bees, mealworms, fish, and frogs.

*Epinephrine.*—The toxicity to mice of oxygen under high pressure is increased by epinephrine, levarterenol, and atropine, and is decreased by adrenalectomy, adrenal demedullation, tryptamine, oxytyramine, or cholinergic vasodilators [Gerschman *et al.* (68)]. Bean & Johnson (69) concluded that in rats the pulmonary edema resulting from exposure to high pressure oxygen is attributable in part to release of epinephrine by a neurogenic mechanism originating possibily in the hypothalamus. A similar mechanism to explain the acute pulmonary edema produced by air blast impact on the head has been suggested by Cassen & Kistler (70).

The ventricular tachycardia produced by epinephrine during cyclopropane anesthesia is caused by, or is coincident with, an increase in arterial plasma potassium. O'Brien *et al.* (71) found that elimination of the hepatic circulation in dogs diminished this rise in arterial potassium and prevented the occurrence of ventricular tachycardia.

During the acute cardiovascular response to intravenous epinephrine in dogs there is a period of marked vasodilation in the femoral vascular bed. An earlier study of this phenomenon suggested that this was attributable partly to passive distention and partly to a reflex mechanism presumably from the carotid sinus (72). Gruhzit, Freyburger & Moe (73) in an extensive study have traced out this reflex. By eliminating all known reflexes and by administration of the epinephrine to selected parts of the arterial system they have drawn the conclusion that mechanoreceptors along the course of the thoracic aorta, activated by the inotropic cardiac action of epinephrine, may be the afferent source of this reflex.

Lee (74) showed that a mixture of tyrosine, niacinamide, and pyridoxine fed to rats does not increase arterial pressure, adrenal weight, or epinephrine content of the adrenals. He could not find any evidence that this mixture served as a precursor for epinephrine. Peltola & Vartiainen (75), making use

[4] The very recent review of the pharmacology of vascular smooth muscle by Furchgott [*Pharmacol. Revs.*, **7**, 183–277 (1955)] covers this point, and many others, in detail.

of the well known fact that thyroid can increase the sensitivity of experimental animals to epinephrine, described a new method of biological standardization for thyroid preparations. Epinephrine, injected intravenously, inhibits bile flow in rabbits [Archdeacon, Danforth & Dummit (76)]. Epinephrine has no influence on the plasma levels of 17-hydroxycorticosteroids in adrenalectomized rats treated with cortisone or intact rats treated with ACTH [Bacchus (77)].

*Levarterenol.*—Romano, Meyers & Anderson (78) found that small doses of the lower aliphatic aldehydes had effects similar to levarterenol in dogs. They suggest that the aldehydes act by combining with normally occurring norepinephrine to form a Schiff's base, and that the vascular response depends on whether the combination is stable or labile. If it is labile, the primary amine is supposedly regenerated to bring about the pressor response. Their conclusions are weakened by several misconceptions about the so-called qualitative differences between epinephrine and levarterenol. In addition, they call upon a nonexistent anticipatory reflex: "... the sympathetic hormone released in response to the threatened hypotension."

Using the Fick method, Delaunois & Vandenberghen (79) studied the effect of single intravenous injections of levarterenol on the cardiac output and metabolism in dogs. The output was increased; the larger the dose, the greater the increase in output. The results must be viewed with great skepticism for two reasons: (*a*) a 1-min. Fick is extremely questionable, and (*b*) they report a stroke volume of 236 ml. in a twenty kg. dog!

Muirhead, Goth & Jones (80) infused massive doses of levarterenol in dogs and followed plasma levels of sodium, potassium, and chloride. There was a fall in plasma sodium that correlated with the rise in arterial pressure. Plasma potassium was elevated, while the chloride was unchanged. Handley & Moyer (81) present evidence to show that with doses of levarterenol sufficient to elevate the pressure above 170 mm. Hg, sodium excretion in the urine is diminished. Lower doses of levarterenol cause an increased urinary excretion of water, sodium, and potassium. They conclude that levarterenol has no direct influence on the absorptive capacity of tubules but can influence this capacity indirectly by changes in renal hemodynamics.

Nickel *et al.* (82) studied extensively the effects of levarterenol on electrolyte excretion in man. Both sodium and potassium excretion are diminished, while water excretion is elevated. Adrenal cortical deficiency does not affect this response. Diminished formation of ADH may account for the increased water output but does not account for the electrolyte changes. High spinal anesthesia did not modify the action of levarterenol, and the pressor response did not appear to be a necessary part of the action. Although their results were different from those of Handley & Moyer (81), they reached the same conclusion, namely that levarterenol indirectly influences electrolyte excretion by changing renal hemodynamics.

Two investigators, using different methods in different species, reached the same conclusions regarding levarterenol and hypertension. Conway (83)

found that hypertensive rabbits are more sensitive to the pressor effect of levarterenol. Greisman (84) found that hypertensive patients as judged by vasomotion of the capillary bed of the nailfold are more sensitive to levarterenol. In a study of the human fetus in prolonged asphyxia, Enhorning & Westing (85) state that levarterenol produces changes in the ECG similar to those found in adults with coronary thrombosis of the anterior wall.

*Adrenergic blockade.*—Drugs affecting the adrenergic neuroeffector mechanism in chickens, particularly the adrenergic blocking agents, have been extensively studied by Harvey *et al.* (86). Epinephrine "reversal" can be produced by phenoxybenzamine (Dibenzyline, N,N-phenoxyisopropylbenzyl-$\beta$-chloroethylamine). Isoproterenol can produce a depressor response. Other adrenergic blocking agents are less effective than phenoxybenzamine in producing epinephrine "reversal" or blockade of sympathetic reflexes or sympathoadrenal discharge. Angiotonin may be pressor or depressor in chickens; the pressor action can be "reversed" by phenoxybenzamine. From the results obtained by these workers one must conclude that the chicken is very similar to the rabbit in its responses to adrenergic drugs.

Leitch, Liebig & Haley (87) have described the use of the isolated seminal vesicle of the rat to assay adrenergic blocking activity. This preparation appears useful since it gives a linear log-dose response to epinephrine, is more sensitive to epinephrine than acetylcholine, and is quite insensitive to histamine. Levy, North & Wells (88) produced traumatic shock in rats by tumbling and found that adrenergic blocking agents administered prior to the tumbling increased the mortality during tumbling but significantly decreased total mortality. It is their conclusion that sympathetic discharge is a protective mechanism during trauma but initiates other processses that are ultimately detrimental.

Adrenergic blockade of only one kidney has been accomplished by intra-arterial injection in dogs by Handley & Moyer (89). Although blockade of epinephrine or levarterenol constriction was present, no evidence for a tonic sympathetic effect on the renal blood supply was found. In man, Moyer, Snyder & Miller (90) studied the effect of adrenergic blockade by phenoxybenzamine on the cerebral blood flow in hypertension. Cerebral blood flow in general tended to follow the arterial pressure; no evidence of a preferential release of adrenergic cerebral vasoconstriction was found.

The peculiar depressor agent, hydralazine hydrochloride (Apresoline; 1-hydrazinophthalzine hydrochloride) has been studied primarily as a therapeutic agent. For example, Rowe *et al.* (91) found it to be a coronary dilator in hypertensive patients, and Finnerty (92) recommends it for the management of hypertension in pregnancy. Stunkard, Wetheimer & Redisch (93), on the basis of evidence obtained in patients, believe hydralazine to be a direct vasodilator rather than an adrenergic blocking agent. Tripod & Meier (94) using only isolated hind limbs of rabbits and the perfused mammalian heart propose a complicated theory of "master-receptors" to explain the actions of hydralazine and related therapeutic agents. On the whole,

there is little evidence to support the idea that hydralazine is an effective adrenergic blocking agent.

The comparative effects of the optical isomers of a new adrenergic blocking agent, Dibozane [1,(4-benzodioxan-2-methyl) piperazine] have been studied in hypertensive patients by Kramer, Horne & Bellet (95). The levorotatory isomer was the most active vasodepressor. However, its side effects, cardiac arrhythmia, drowsiness, nausea, vomiting, and others, were quite severe. The meso-isomer produced changes in cerebration while the dextroisomer was more or less inactive. Harvey & Nickerson (96) present evidence that the $\beta$-haloalkylamines act as adrenergic blocking agents by alkylating sulfhydryl groups.

*Chlorpromazine.*—Chlorpromazine [10-(3-dimethylaminogropyl)2-chlorophenothiazine], a widely used therapeutic agent, has had an interesting and brief history. Clinical studies, many poorly controlled, have completely overshadowed any investigations as to its real physiological effects. Its actions are so complex and manifold that many of them seem to have been overlooked in the clinical trials. Moyer *et al.* (97) have pointed out some of the precautions to be observed in patients. Chlorpromazine is a direct vasodilator and a fairly active adrenergic blocking agent but has no peripheral cholinergic blocking action (98). Kopera & Armitage (99) were unable to demonstrate adrenergic blockade against levarterenol. Chlorpromazine can protect the ventricle from hydrocarbon-epinephrine arrhythmias [DiPalma & Catenacci (100)]. It can protect mice from x-irradiation [Haley *et al.* (101)]. Holzbauer & Vogt (102) found that chlorpromazine did not prevent the stimulant action of morphine on central sympathetic centers. They also found that chlorpromazine did not affect the release of ACTH by trauma or epinephrine and that it had no ganglionic blocking action in the superior cervical ganglion. They did, however, find it to be an adrenergic blocking agent.

*Veratrum alkaloids.*—The alkaloids from veratrum and related compounds are of interest because of their manifold cardiavascular effects. Most of these effects are reflex in nature, and these are considered elsewhere in this volume. Some of the actions, however, are more direct, either on the central nervous system or on various effector organs.

In man, protoveratrine administered intravenously reduces cardiac output, cardiac work, and, occasionally, the total peripheral resistance [Hoobler, Kabza & Corley (103)]. The most interesting finding by these authors was that urine flow decreased markedly while renal blood flow and glomerular filtration rate were relatively unchanged. Blackmore (104) studied the effect of protoveratrine on urine flow in normal dogs and those with diabetes insipidus. In the normal dogs urine flow decreased, while in the diabetic dogs there was no change in urine output. This was taken as evidence that protoveratrine stimulates the release of ADH. Protoveratrine injected intravenously in vagotomized dogs produces a marked, but transient, increase in secretion of epinephrine from the adrenal medulla [Aronow & Howard (105)].

Veratramine antagonizes the positive chronotropic action of epinephrine or levarterenol. Krayer & Ourisson (106) have found that veratramine also antagonizes the positive chronotropic action of ephedrine, phenylephrine, tyramine, and isoproterenol. Veratramine also antagonizes the cardioaccelerator effect of sympathomimetic agents in the presence of atrioventricular rhythm produced by clamping the sinoatrial node in intact dogs [Krayer, Arora & Meilman (107)]. Veratramine can produce asystole in the presence of atrioventricular rhythm, which it does not do in the presence of sinoatrial rhythm. It is the conclusion of these authors that veratramine, in doses that are strongly antiaccelerator, possesses little quinidinelike antiarrhythmic action. Kosterlitz, Krayer & Matallana (108) have described a peculiar periodic rhythm of the denervated heart of the spinal cat produced by veratramine. The initial heart rate of about 200 beats per min. decreases rapidly to about 50. Then follow periods of complete asystole alternating with periods of activity of a frequency between 90 and 140 beats per min. During the time of periodic rhythm, infusion of epinephrine, or stimulation of postganglionic accelerator fibers, either shortens the periods of inactivity or restores a regular rhythm. If epinephrine is infused, or the nerve stimulated, after the periodic rhythm has changed to a regular rhythm, the peroidic activity is restored.

Although there can be little doubt that the veratrum alkaloids produce bradycardia by decreasing sympathetic activation of the heart, it may be that this effect is a reflection of the direct antiaccelerator action of these alkaloids (106). Wang, Ngai & Grossman (109) showed that veratrum alkaloids injected into the adventitia of the carotid sinus produce hypotension in vagotomized dogs. This hypotension, a reflex attributable to repetitive firing of the baroreceptors, results from inhibition of the sympathetic nervous system since the hypotension is prevented by sympathectomy or adrenergic blockade. Moran, Perkins & Richardson (110, 111) found similar results as far as the fall in arterial pressure is concerned.

*Central cardiac effects.*—Berman, Simonson & Heron (112) induced hypnotically an anxiety-fear situation and a work-pain situation in normal subjects and in patients with coronary sclerosis and angina pectoris. Various electrocardiographic changes appeared in some individuals in both groups. This study shows that emotional states as well as organic heart disease can produce abnormal electrocardiograms.

Another interesting study showing that the central nervous system can mediate certain peripheral cardiac effects is that of Weinberg & Haley (113). These workers injected various drugs into the third cerebral ventricle of trained, unanesthetized dogs. Strophanthin, in doses that had no cardiac effect when given intravenously, produced marked cardiac arrhythmias including bigeminy, bradycardia, and paroxysmal ventricular tachycardia. Quinidine produced atrial and ventricular tachycardia. The effects of strophanthin could be prevented by quinidine or procaine amide injected into the cerebral ventricle or by hexamethonium injected intravenously.

*Acethylcholine.*—Evidence continues to accumulate indicating that acethylcholine acts as a local hormone. Isolated rabbit hearts perfused with Locke's solution containing physostigmine form acetylcholine which can be identified biologically in the perfusate [Briscoe & Burn (114)]. Cocaine does not affect this formation. However, arresting the heart with calcium-free Locke's solution, or by adding a trace of dinitrophenol, diminishes the formation of acetylcholine. Burn & Walker (115), in the canine heart-lung preparation, have evidence that acetylcholine is being continuously formed. If the vagi are sectioned four days prior to making the heart-lung preparation, acetylcholine is still formed. Drugs which inhibit pseudocholinesterase slow the heart rate in this preparation. Atropine then causes the initial heart rate to return. Inhibition of true cholinesterase does not affect the heart rate. The contractions of the isolated rabbit atria are arrested by the addition of small amounts of acetylcholine in the presence of low concentrations of anticholinesterase drugs [Tedeschi (116)]. Some anticholinesterases, physostigmine, and neostigmine for example, act like atropine in causing the arrested atria to start contracting again. The organic phosphates such as DFP do not restore contraction. Long-acting, relatively irreversible anticholinesterases when administered by close arterial injection can induce spontaneous secretion of saliva by the submaxillary gland in cats and dogs. This spontaneous flow is not affected by nerve section or degeneration but is slowed, but not stopped, by ganglionic blockage. On the basis of this and other evidence, Dirnhuber & Evans (117) believe acetylcholine to be formed constantly in the gland.

Pilgrim (118) has examined the effects of graded concentrations of acetylcholine on isolated ventricle strips of 10 species of bivalve molluscs (lamellibranches). Some species were inhibited with diastolic arrest occurring at high concentration. Other species showed increased activity with final systolic arrest.

Stimulation of the vagus nerve in frogs causes the heart treated with physostigmine to contain more acetylcholine. Anand (119) has found this acetylcholine formation to be temperature dependent. More acetylcholine is liberated as the temperature increases, up to 40°C. Holtz & Westermann (120) found that propionyl and butyryl choline have two actions on the isolated guinea pig heart: one is a typical acetylcholine-like inhibitory effect that is prevented by atropine; the other is an adrenergic stimulation that can be prevented by hexamethonium. In addition these two esters can restart the arrested rat heart.

Substances that produce contraction of isolated segments of mammalian intestine can act either by stimulating the postganglionic cholinergic neuron at the synapse or by stimulating the muscle cells directly. Ambache & Lessin (121) have developed a method for separating these two mechanisms of action. Exposure of the intestinal segment to a purified botulinum toxin paralyses the postganglionic neuron. Nicotine can then no longer produce contraction, while acetylcholine or muscarine is still active. Using this

preparation these authors have tested numerous drugs. On the guinea ileum, for example, barium chloride and potassium chloride appear to act primarily on the postganglionic neuron. On rabbit ileum the actions of histamine and physostigmine are apparently also neuronal.

Another approach has been used by Parkes & Sacra (122) to differentiate sites of action of drugs. They compared the antidotal effects of atropine, tubocurarine, and hexamethonium against four anticholinesterases. Different effects were obtained, indicating that both the distribution and the mode of action of anticholinesterases may vary with changes in dose level.

*Cholinesterases.*—Cavanagh, Thompson & Webster (123) believe pseudocholinesterase to be localized in the glial cells of the central nervous system. In the peripheral nerves this enzyme is not associated with any conductive elements. Koelle (124) has recently extended his observations on the localization and characteristics of the various cholinesterases. Of particular interest is the finding that there are cholinesterases that are resistant to the selective inhibitors of acetylcholinesterase or pseudocholinesterase. These resistant enzymes are found principally in the brain. Acetylcholinesterase was found not only in peripheral cholinergic neurons but also in all adrenergic and sensory neurons examined. Koelle suggests that his evidence is consistent with a theory that neurons may liberate mixtures of transmitters and that the terms cholinergic and adrenergic should refer to the predominant rather than exclusive transmitting agent.

Hexamethonium chloride (or bromide) has replaced TEA as the prototype ganglionic blocking drug. In thirteen hypertensive patients an intramuscular dose of hexamethonium, sufficient to lower mean arterial pressure 39 per cent, reduced cerebral blood flow 16 per cent and cerebral vascular resistance 29 per cent according to Crumpton *et al.* (125). In patients with essential hypertension, hexamethonium decreased the cold pressor response [Windesheim, Roth & Gifford (126)]. Windesheim, Roth & Hines (127) also found that hexamethonium was not an effective peripheral vasodilator in severe arteriosclerotic obliterans. Although the drug reduced the arterial pressure in these patients, no apparent dilation occurred in the arteriosclerotic areas. Burch (128) showed that hexamethonium lowers venous pressure in congestive failure of the circulation.

Perry & Reinert (129) have found that hexamethonium potentiates, rather than blocks, the stimulating effects of acetylcholine on the perfused, chronically denervated, superior cervical ganglion of the cat. Hexamethonium itself stimulated the ganglion cells. The innervated ganglion responded in the same manner if perfused with low-potassium Locke's solution. In denervated ganglia perfused with high-potassium Locke's solution or with a solution containing sodium glutamate, hexamethonium blocks the effects of acetylcholine. Other ganglionic blocking agents tested by the authors, tubicurarine, atropine, and TEA for example, blocked the actions of acetylcholine in all preparations. These authors have also demonstrated that cardiac glycosides in small doses potentiate and in large doses depress the effects of acetyl-

choline on the superior cervical ganglion of the cat (130). The potentiating effect is abolished by the absence of potassium. The glycosides also potentiate the effects of acetylcholine on the isolated mammalian heart; this potentiation is prevented by hexamethonium.

Exley (131) examined the effects of barbiturates on autonomic ganglia in an attempt to explain the well-known depressor effects of these agents. He compared the anesthetic potencies of the barbiturates in mice with their effects on vasomotor ganglia (pressor response to nicotine) and their effects on the superior cervical gaglion in cats. The principal results were as follows: (*a*) barbiturates produce transient depression of ganglia at the synapse, (*b*) barbiturates reduce excitability of ganglion cells to acetylcholine but not potassium, (*c*) changes in chemical structure of barbiturates change ganglionic depressant activity, and (*d*) ganglion depressant potencies show little correlation with central nervous system depressant potencies. It is definitely shown that the depressor action of the barbiturates is attributable in part to ganglionic blockade.

Atropine can produce postural hypotension in man because of ganglionic blockade [Kalser, Frye & Gordon (132)]. Compression of the abdomen and lower extremities prevents the vascular pooling of blood resulting from atropine ganglionic blockade [Miller *et al.* (133)].

TEA or hexamethonium when injected into animals receiving a continuous infusion of levarterenol produces a pressor response [Bartorelli, Carpi & Cavalco (134)]. According to the results of these authors this potentiation of levarterenol occurs only when both the sympathetic and parasympathetic transmissions are blocked. Fredericq (135) has attempted to assess the ganglionic blocking effect of histamine and the antihistaminic, pyrilamine (Neo-Antergan). He used extremely high concentrations of the drugs and tested the effect of stimulation of the cardiac pneumogastric nerve to the turtle left atrium. His conclusions, not well supported by his data, are that pyrilamine is a vagolytic, and anticholinesterase, an anticholinergic and a ganglionic blocking agent.

*Dilator substances.*—Holton & Holton (136) have described in more detail their studies of the dilator substance in dry powders of spinal roots. The dilator activity is due to ATP and ADP. These latter substances when injected arterially in the rabbit ear give a response resembling antidromic nerve stimulation.

Werle & Erdos (137) have extracted from the urine of healthy humans a new depressor substance. This substance also stimulates isolated rat or guinea uterine strips and has some antidiuretic properties. It appears to be a hitherto undescribed polypeptide.

It has long been known that activation of the submandibular salivary gland produced by stimulation of the chorda tympani is accompanied by vasodilation in the gland. This vasodilation is not completely abolished by atropine. Hilton & Lewis (138) have made an extensive study of this vasodilation. Botulinum toxin abolished both the salivation and dilation, indicating

that cholinergic nerves are involved. By comparing the effects of occluding the arterial and venous flows on the vasodilation, the authors conclude that a stable vasodilator substance, resistant to atropine, is produced in the activated gland.

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# HIGHER FUNCTIONS OF THE NERVOUS SYSTEM[1,2]

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During the past year there have been several important developments in our understanding of the higher functions of the nervous system on aspects of this subject not completely covered by the two previous reviewers, Harlow (70) and Lindsley (97). We shall not attempt a comprehensive review. We shall cite only those studies which seem to us to be of particular current interest and importance, including some earlier publications omitted from the reviews of previous authors.

Books which should be cited are Penfield & Jasper's *Epilepsy and Functional Anatomy of the Human Brain* (123), Bremer's *Some Problems in Neurophysiology* (17), Gellhorn's *Physiological Foundations of Neurology and Psychiatry* (58), The Laurentian Symposium entitled *Brain Mechanisms and Consciousness* (2), and Granit's *Receptors and Sensory Perception* (66). In each of these books will be found comprehensive reviews of much work on the higher functions of the nervous system in addition to many stimulating hypotheses which are destined to guide research on this subject for years to come.

## THE RETICULAR SYSTEM

Recent studies of the anatomy and functional properties of the brain stem reticular system have made it necessary to include at least the upper portion of this system, in the diencephalon and midbrian, among those most important for the more highly integrated activities of the nervous system. This was clearly brought out in the Laurentian Symposium (2) which provides an excellent summary of current work on this subject, together with extensive discussion of divergent points of view. [See also a critical review by Cobb (36)].

This symposium was opened by Magoun, who presented a concise but comprehensive summary of the evidence for the critical importance of the ascending reticular system to wakefulness. In summarizing present conceptions of the functional organization of the activating system in the brain stem, Magoun states (2, p. 11)

> Its direct stimulation desynchronized the E.E.G. in a manner simulating awakening from sleep or alerting to attention. Collaterals, from all afferent paths to the cortex turn into this system to be mediated through it. Injury to its cephalic portion leads to chronic loss of wakefulness. Reversible pharmacologic block of its ascending influences upon the hemipshere may provide a neural basis for the anaesthetic state. [See also (48)].

[1] The survey of literature pertaining to this review was concluded in July, 1955.

[2] The following abbreviations will be used: EEG (electroencephalogram); LSD (lysergic acid diethylamide).

There was some question, however, as to whether one was justified in identifying the so-called "desynchronization" of the EEG with wakefulness in all instances and as to whether all parts of the reticular system were uniformly effective in arousal.[3] Morison questioned the inclusion of the intralaminar system of the thalamus with the subthalamic, hypothalamic, and mesencephalic portions of the reticular system, which may have different functional properties. There remained also the question of whether the reticular system had only a gross energizing action upon the cortex as a whole, or whether more highly integrative differential functions could be ascribed to it.

Moruzzi, in summarizing the work from his laboratories using microelectrodes to record unit responses in the reticular system, pointed out that impulses from the cerebellum also coverge upon reticular neurons and may cause changes in cerebral cortical electrical activity similar to that in arousal, but he warned against assuming the identity of all processes which produce a similar change in the EEG from the cortical surface, since microelectrode studies may show quite different conditions existing in the discharge of individual cortical neurons. Moreover, inhibition of reticular cell discharge may also occur in response to cerebellar and cerebral cortical stimulation, as well as in response to sensory volleys. In some instances a given reticular unit is excited by a sensory volley but inhibited by cerebellar stimulation. Convergence of effects from different sources was found on the same units, as noted also by Amassian and Devito (5) but such convergence was not unlimited. There was evidence from some overlapping topographic organization with reference to various sensory modalities (147). Moruzzi expressed the belief that the massive all-or-none character of reticular system response may be attributable to our gross methods of study, and that a fine differentially organized function of both excitatory and inhibitory character may be the more physiological nature of the normal function of the reticular system. This would provide for more highly integrative functions such as in the direction of attention.

This conception would be in accord with that proposed by Jasper (2) in his discussion of the thalamic reticular system, which can be shown to have a definite topographical organization in respect to cortical areas of projection, and to possess functional properties distinct in several respects from those of the more caudal portions of the reticular system.[4] The question of

[3] Gastaut, (2, 249) pointed out important relationships between the EEG and states of consciousness in patients with brain stem lesions involving the reticular formation, with some interesting discrepancies between the EEG and clinical evidence which emphasizes the error in identifying the electrical activity of the cortex with functional states of consciousness [see also Pond (125)].

[4] Jasper reviewed his work with Li showing with microelectrode analysis of the recruiting response that "unspecific" afferents from the thalamic reticular system are distributed differently in the cortex than are the majority of specific afferent terminals. It was suggested that they may terminate on dendrites to regulate excitability of the

unspecific afferent projections to sensory areas of cortex has since been settled in the affirmative by Jasper, Naquet & King (78).

A more highly differentiated system is also suggested from the cytoarchitectonic studies presented by Olszewski, who concludes that

> the reticular formation cannot be looked upon as a morphological unity, or as a sort of ill-defined network in which, here and there, some "nuclei" formed by accumulation of nerve cells can be recognized. Instead, the reticular formation must be considered as a collection of nuclei of very different structure. Therefore it is our opinion that the term "reticular formation" could advantageously be dropped from both anatomical and physiological paralance . . . an effort to correlate physiological observations with the arrangement of nuclei and fibre tracts should prove much more fruitful than the present day practice of regarding the reticular formation as an anatomical entity. (2, p. 74)

Although the ability to distinguish morphological "nuclei" does not necessarily imply specificity of function, it is clear that one cannot attribute functional identity to a sytem of neurons just because they have in common the property of awakening an animal from sleep or of producing what appears grossly to be a similar effect upon the electrical activity of the cortex. However, the extensive interconnected system of neurons functionally defined as "reticular system" does not imply morphological or functional identity of all its parts. This point of view is emphasized by Gellhorn (58, 59), who lays emphasis upon the hypothalamic portion of the activating system.

A far more comprehensive view of the significance of the reticular system is presented by Penfield in his hypothesis of a centrencephalic integrating system (123), based upon extensive studies of the effects of lesions and electrical stimulation of the brain in conscious man. He states that (2, p. 286)

> an area of final functional integration must exist—a ganglionic area in which that stream of nervous impulses must arise that produces voluntary activity, an area in which the sensory pathways culminate in neurone circuits and in which information relative to past experience is made available, an area in which those nervous mechanisms are to be found which are prerequisite to the exictence of intellectual activity and prerequisite to the initiation of the patterned stream of efferent impulses that produce the planned action of the conscious man.

Fessard (2, p. 225), in a brilliant discussion of the neurophysiological evidence and "logic" of brain function in relation to conscious experience conceives of the reticular systems as integrators of the highest form where "convergence of volleys from heterogeneous origins towards a well-defined polysynaptic field is the complex activity that corresponds to the highest form of nervous integration."

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specific synaptic systems. This introduces the important subject of dendritic function of the cortex in integrative processes which cannot be discussed because of lack of space (33, 34, 161).

The demonstration that many parts of the cerebral cortex have more or less direct projections to the reticular system of the thalamus and brain stem (3, 20, 21, 22, 47, 79) and that these same areas are capable of arousing an animal from sleep helps to complete the picture and make more convincing the conception that neuronal circuits in the core of the brain stem may have important integrative functions for more complex activities of the brain as a whole. Lashley (2), however, was unimpressed by this evidence and these arguments and expressed the opinion that we still must look to the more dynamic functional properties of the cerebral cortex itself, not to the brain stem, for the highest level of nervous integration, a view shared by Bremer *et al.* (2, 18, 19). It is apparent that it will be only through a more thorough understanding of mechanisms of interaction between cortex and brain stem that this important problem will be further elucidated.

The recent demonstration that the reticular system exerts centrifugal effects directly upon sense organs (50, 65, 66, 67, 72, 73, 83) adds another interesting chapter to the story of central integrative mechanisms of the reticular formation. It seems likely that from a closely interconnected neuronal network in the upper brain stem not only can the excitability and temporal characteristics of cortical activity be regulated, but descending influences may be exerted upon afferent inflow through regulation of the response of sense organs. This parallels effects upon motor systems by regulation of muscle tone and excitability in segmental reflex centers. It has been recently shown that these efferent effects may also be organized in a reciprocal manner in relation to antagonistic muscle systems (60), which implies a more coordinated function within the system, even at bulbar levels, than was apparent from initial studies.

Another approach to the problem of the integrative function of the reticular system has been carried out by Sharpless (157) making use of rapid adaptation of reticular responses to repeated identical stimuli. Sleeping cats were initially aroused by any sound, but upon repetition of a tone at a given frequency there was no longer any response to this tone, though another tone, if administered for the first time, had still retained its arousal capacity. Adaption to a specific tone did not impair response to other forms of stimulation (touch or light). This frequency and modality-specific adaptation or habituation, if overtrained, would persist for several days without reenforcement. Complete excision of auditory and somatic sensory receiving areas, together with the suprasylvian gyrus in the cat, was without effect upon the frequency specific habituation. With the use of repetitive click stimuli, habituation of the arousal system occurred readily, without appreciable change in evoked potentials from the auditory receiving areas. It seems clear, therefore, that considerable discrimination in response of the reticular arousal system could be established even without the cerebral cortex. It was not possible, however, to establish habituation which was specific to a given tonal pattern, independent of frequency differences, in animals with large cortical excisions. This would suggest that the more complex discrimination

of temporal patterning requires the corticofugal projection system. It is hoped that these studies will be continued to include positive conditioning of stimulus patterns, for it may be an important approach to the problem of how stimuli gain "significance" or attentive value for behavior, and may provide a neurophysiological basis for one of the most important integrative functions of the brain. (An important discussion of this problem in relation to perception is provided by Adrian (1, 2, p. 237).)

## THE RHINENCEPHALON

Among the neurophysiological investigations of recent years the experimental work on the rhinencephalon or limbic system has given rise to much interest and speculation, especially in view of the possible importance of this system for the integration of emotional behavior.

Physiological studies on the limbic system have always shown that a remarkably wide spectrum of activities is apt to be influenced by rhinencephalic structures. In this respect the rhinencephalon shares the characteristics of "unspecificity" with the reticular system of the brain stem.

### Elementary Somatic and Autonomic Functions

Previous studies by Ward (170), Kaada (80), Gastaut (53), MacLean & Delgado (99), Koikegami *et al.* (85 to 90), and many others have shown that stimulation within the rhinencephalon is able to produce a variety of somatic and autonomic responses; the very fact that they encompass such a wide range of activities excludes the possibility that the rhinencephalon specifically regulates any well-defined single elementary function as, e.g., respiration, digestion or locomotion, although obviously all these functions are apt to be influenced by rhinencephalic stimulation.

It is not within the scope of this review to summarize again the earlier evidence bearing on this "unspecific" character of rhinencephalic stimulation responses. Excellent reviews on this subject have been written by Kaada (80), Fulton (49), Gastaut (53), and more recently by MacLean (98) and Pribram & Kruger (130).

The results of these previous studies have been confirmed by recent investigations and further evidence for similar effects in man has been added [Kaada, Andersen & Jansen (81, 82); Koikegami and co-workers (89, 90); Chapman *et al.* (25); Baldwin *et al.* (9); Pool (126); Segundo *et al.* (153); Austt *et al.* (7); Cadilhac (24)].

### Topographical Organization

It has always been a puzzle to many to understand the absence of good evidence for a topographical localization of specific functions within this system. In recent studies Kaada, Andersen & Jansen (82) were the first to establish a functional subdivision of the amygdala, in which they distinguish two main functional areas. According to these authors the more elementary somatomotor effects (contraversive movements, tonic-clonic movements of

the extremities, licking, sniffing, chewing, and inhibition of respiratory and other spontaneous somatomotor activities) as well as the autonomic responses (pupillodilatation, salivation, micturition, defecation, and piloerection) are represented in the medial portion of the amygdaloid complex, whereas the lateral part would elicit mainly behavioral responses suggestive of attention, fear, or rage—responses which this lateral part of the amygdala seems to share with the neighboring hippocampus and with all the medial cortical areas of the limbic lobe surrounding the hilus of the hemisphere [Kaada, Andersen & Jansen (82)]. It is however doubtful whether, on the basis of the map they present, one can accept their conclusions without reservations, since the most striking fact seems to be the extensive overlap of all sorts of responses, especially in the region of the basal nucleus. The same type of overlap in the same area is found in the work reported by Koikegami *et al.* (85 to 90).

## Neuroendocrine Mechanisms

Recent investigations have shown that the rhinencephalon is related to the mechanisms of neuroendocrine integration. In view of the wide spectrum of somatic and autonomic functions activated by rhinencephalic stimulation the extension of this spectrum into the endocrine field could almost be predicted. The actual demonstration of this relationship started with the work of Schreiner & Kling (149). These authors demonstrated that marked hypersexuality develops in cats after bilateral removal of the amygdala and of the overlying mediobasal temporal cortex. This has been observed in several other animal species as well (150). This release of sexual drive was suggestive of a functional derangement in the hypothalamopituitary control of gonadal functions. In a later study Schreiner & Kling (151) actually demonstrated a hormonal link in the chain of events leading to hypersexuality in these amygdalectomized animals, since castration was followed by disappearance of the abnormal sexual behavior. From these studies one must assume by inference that the amygdala inhibits gonadotrophin secretion by the pituitary in acting on hypothalamopituitary mechanisms.

However, other experimental studies demonstrated that the amygdala can exert an activating influence upon this system as well, thus adding another example to the list of potential dual effects of rhinencephalic activity upon visceral and somatic functions [e.g., topographical overlap of points producing increase and decrease of arterial pressure and of points facilitating and inhibiting somatomotor functions as described in Kaada's monograph (80)]. Koikegami *et al.* (89) produced ovulation in the female rabbit by amygdaloid stimulation. This response was almost constant during estrus but still occurred sometimes in the anestrus phase of the cycle. Additional evidence that the rhinencephalon is involved in the rabbit's ovulation mechanism is given in a recent study by Sawyer (146), who found that histamine injections into the third ventricle, when given under light pentobarbital anesthesia activates rapid electrical activity in rhinencephalic structures and

along their projections to the hypothalamus, especially in the preoptic region and around the medial forebrain bundle. Under these experimental conditions a female rabbit, brought into estrus by previous estradiol benzoate injections, will ovulate; it will not ovulate, however, if the rapid activity in the rhinencephalic structures is blocked by atropine injection or if the areas giving rise to this activity are destroyed surgically.

The endocrine functions of the rhinencephalon, however, do not seem to be restricted to the sphere of gonadotrophin secretion. Koikegami & co-workers (90) report on histological studies performed on endocrine glands after bilateral destruction of the amygdaloid nuclei in puppies. Growth was markedly slowed in these young animals, suggesting some interference with hormonal mechanisms of growth. The endocrine glands in these amygdalectomized puppies showed marked atrophy. This was very apparent in the pituitary gland, especially in its anterior lobe. There was also atrophy and signs of colloid retention in the thyroid gland, decrease of Langerhans' islets in the pancreas, and atrophy of the adrenal cortex, especially of the zona fasciculata. Unfortunately no such studies on adult animals have been reported. From the lack of any severe "clinical" endocrine syndrome in adult animals after similar lesions, one would, however, suspect that the endocrine changes would not be as profound as they are reported to be in the infantile organism.

### Relationship with Subcortical Structures

Important advances have been made recently in the field of electrophysiology of the rhinencephalon, especially in connection with the problem of its relationship to brainstem and other subcortical mechanisms.

Green & Arduini (68) made extensive studies of the electrical activity of the hippocampus in relation to arousal. Their findings have recently been confirmed by Liberson *et al.* (95, 96), by Cadilhac (24), and by Faure & Portmann (44). They describe the normal resting pattern of the hippocampus and its modifications by arousal as opposed to the correlated electrographic patterns in the neocortex. There is a striking inverse relationship between patterns recorded in the hippocampus and in the neocortex during identical states. Thus the relaxed or drowsy state which is characterized in the neocortex by synchronized activity, often appearing in spindles, is accompanied by desynchronized activity in the hippocampus. The basic frequencies of this desynchronized hippocampal activity are, however, considerably slower than the frequencies of the desynchronized neocortex during arousal. During arousal by any sensory stimulus the hippocampus shows synchronization with occurrence of rhythmical 3 to 7 per sec. slow waves, whereas the neocortex displays an asynchronous fast pattern. This pattern is most clearly seen in rabbits, somewhat less clearly in cats, and is the least prominent in monkeys. This hippocampal arousal pattern can also be reproduced by stimulation of the brain stem reticular formation, the posterior hypothalamus, the intralaminar nuclei, the preoptic region, and the septal area. There is

considerable adaptation of the hippocampal arousal response to repeated identical arousal stimuli. Hippocampal and neocortical arousal are relatively independent, though they usually occur together.

Lesion experiments suggest that this hippocampal arousal response is induced in the hippocampus from the brain stem and mediated via the hypothalamus to the septum and thence to the hippocampus by way of the precommisural or dorsal fornix. Subcortical records suggest that the rhythmical pattern in turn is projected from the hippocampus back into the subcortex, especially into the mammillary region and the region of the habenulo-interpeduncular tract via the fornix proper. The functional significance of these findings is somewhat open to debate. The unusual degree of alertness of animals with septal lesions involving the fornix columns, as reported by Brady *et al.* (14), could indicate that hippocampal arousal activity may exert an inhibitory influence upon the mammillary region and the posterior hypothalamus. These areas in turn are known to produce neocortical and behavioral arousal.

At this point it might be of interest to mention observations reported by Liberson & Cadilhac (95) and Cadilhac (24) that the hippocampal evoked potentials to auditory and visual stimulations described previously by Robinson & Lennox (139) are usually seen only during the first few stimuli and then tend to disappear. Responses to visual stimuli often cannot be elicited unless the following procedure is used: During a stimulation period, where clicks and light flashes are delivered in regular alternating succession, the click is suddenly suppressed. This produces a clear visually evoked potential in response to the first few flashes delivered after this sudden change in stimulation pattern. It is as if the hippocampus would only react in a situation where the sensory message carries an element of unexpectedness or surprise and then quickly adapts. This, together with the fact that sensory stimuli often are followed by the rhythmical hippocampal arousal pattern, would be compatible with the concept that the hippocampus may act as an inhibitor of brain stem arousal mechanisms. Further clarification of this relationship is, however, needed.

Green & Arduini (68) finally discuss the possibility of some relation of the hippocampal arousal pattern with the bilateral theta rhythm described by Walter (169) in the human EEG, which, according to this author, bears in some manner on emotional phenomena.

In relation to this problem of hippocampal arousal mechanisms it might be worth while to mention parenthetically that Chatfield & Lyman (26) were able to demonstrate that the hippocampus and its subcortical projections, together with other parts of the rhinencephalon, seem to play a leading role in the arousal of the golden hamster from hibernation. The neocortex, silent during hibernation, apparently becomes aroused in the wake of increased activity originating in rhinencephalic structures.

Green & Arduini's (68) observations prove that desynchronized activity may not be the expression of arousal in all brain regions, but rather represents the particular arousal pattern of the neocortex. This is corroborated

by observations on the amygdala by Feindel & Gloor (45), which showed that stimulation of the brain stem reticular formation evokes a fast rhythmical and synchronized activity at about 16 to 20 per sec. in the amygdala which can also be produced by olfactory stimuli. This pattern is probably identical with the "olfactory-like" responses in the piriform lobe described by MacLean *et al.* (100) and may represent the specific arousal pattern of amygdala and piriform cortex.

However, another aspect of the close relationship of the rhinencephalon to the "arousal" system has been made the main subject of this experimental study by Feindel & Gloor (45). A comparative investigation of electrocorticographic changes induced by amygdaloid and brain stem reticular stimulation in cats showed a surprising similarity in electrographic effects from both loci of stimulation, though the responses were somewhat more labile when produced from the amygdala. Both amygdala and reticular formation on stimulation were able to produce a fast low voltage electrocorticogram, to suppress cortical slow waves and strychnine spikes (provided the strychnine concentration used was liminal), and to produce identical patterns in the amygdaloid electrogram. These findings seem to indicate a close functional relationship between amygdala and brain stem reticular formation.

A subsequent study by Gloor (63) furnished more evidence for the close connection of the amygdala with the brain stem reticular formation and, furthermore, demonstrated the very intimate connection of the amygdala with other subcortical structures as well.

Evoked potentials to single shock and repetitive stimulation showed that the amygdala is able to activate a large subcortical area extending from the septum and the base of the caudate nucleus through the preoptic region, the anterior and posterior hypothalamus, the subthalamus, some of the midline and intralaminar nuclei of the thalamus, down to the tegmentum of the mesencephalon. The first synaptic relays seem to be located in the base of the telencephalon medium and in the adjacent parts of the rostrobasal pole of the diencephalon, whereas all other points within the subcortical amygdaloid projection system are reached over multisynaptic relays. Direct cortical projections relay from the amygdala to the temporoinsular cortex and to the overlying piriform cortex, and a powerful multisynaptic route carries impulses from the amygdala to the hippocampus most probably via neurons in the cortex of the piriform lobe.

The interest of this "electroanatomical" study for the physiologist lies in the fact that it makes more understandable the means by which stimulation of the amygdala is able to influence the wide spectrum of autonomic, somatic, behavioral, and electrographic activities demonstrated by several authors in previous and recent studies. Amygdaloid impulses impinge upon wide multisynptically organized subcortical fields known to regulate highly integrative functions of the nervous system as shown by numerous classical studies from the schools of Hess, Ranson, and Magoun.

The second part of this study on amygdaloid connections by Gloor (64)

deals with the more subtle electrophysiological changes occurring in this system under the influence of repetitive stimulation of the amygdala. These changes are such as to suggest that the amygdala acts in a highly flexible and modulating way upon its subcortical projection areas. Repetitive amygdaloid stimulation induces recruitment in many parts of the amygdaloid projection system. After discontinuation of repetitive stimulation very often a marked post-tetanic potentiation effect is observable, even after repetitive "tetanic" stimulation at frequencies as low as 10 c.p.s. These changes are most prominent in the hippocampus; potentiation, but not recruitment, is also very prominent in the ventromedial nucleus of the hypothalamus. Latency changes in either direction may accompany these changes in excitability. The more a response is multisynaptically relayed, the more pronounced are these dynamic changes induced by repetitive stimulation.

In view of these findings the author believes that the amygdala and perhaps also other parts of the rhinencephalon act as a modulator of complex somatic and autonomic mechanisms integrated in the central core of subcortical basal grey matter extending from the septum through the hypothalamus into the brain stem tegmentum.

### Rhinencephalon and Behavior

The picture of a close though flexible relationship between rhinencephalon and the highest integrative formations of diencephalon and brain stem thus emerges and immediately suggests that the function of these circuits may be important for integrating highly organized behavioral patterns.

Important studies in this field have been published in the past years all enlarging on previous evidence for the importance of rhinencephalic structures for the elaboration of complex behavioral mechanisms. Application of the newer, rigidly controllable techniques of experimental psychology to this field have been very helpful in basing previous behavioral work on firmer ground.

*Spontaneous activity.*—Brady & Nauta (14) observed marked changes in spontaneous activity in rats with septal lesions. They also noted explosive startle reactions, fear on presentation of innocuous objects, aggressiveness, and proneness to escape. These changes were greater with septal lesions involving the columns of the fornix. Similar results were reported by Soulairac *et al.* (159). In a clinical study Gastaut *et al.* (56, 57) showed that discharging lesions in the rhinencephalon, as seen in psychomotor epilepsy, produce the opposite syndrome in most of the patients. These epileptics show slowness of spontaneous behavior and ideation together with slight slowing of background cerebral rhythm, poor driving of the parieto-occipital rhythm of the EEG by intermittent photic stimulation, and a high metrazol threshold for eliciting generalized seizure activity, a syndrome which the authors like to refer to as neuronal hypoexcitability. It might be opportune here to recall Green & Arduini's (68) theory, outlined above, that the hippocampus may act as an inhibitor of subcortical arousal mechanisms integrated in the brain stem reticular formation.

*Emotional behavior.*—An important group of the behavioral investigations was concerned with the study of conditioned "emotional" mechanisms. Brady & Nauta (14) experimented with septal lesions in rats which produced a loss of the conditioned emotional response acquired before operation. Cingulate lesions did not produce this loss. The acquisition of the response was, however, not disturbed after these septal lesions. The effects were most pronounced when lesions involved the fornix columns. In a later study, Brady, Schreiner, Geller & Kling (15) studied the conditioned avoidance response in cats with bilateral lesions of the amygdala. In these animals the acquisition of the conditioned avoidance response was highly impaired, though retention of a preoperatively learned response was not affected by the bilateral amygdaloid lesion. The situation was again different in orbitofrontal removals, where the animals did not retain a preoperatively acquired conditioned avoidance response and also failed to relearn it. Yet, when confronted with the conditioned stimulus, they showed evidence of a conditioned emotional behavior manifested by autonomic changes. These changes are identical to those seen in normal cats trained for the acquisition of the conditioned avoidance response at a time when the significance of the unconditioned stimulus, which signalled an impending unpleasant experience, apparently was already recognized but avoidance behavior had not yet been learned. In these orbitofrontal operated animals, therefore, the emotional significance of the unconditioned stimulus was apparently learned or retained. The way to respond by avoiding the unpleasant experience, however, could not be retained or relearned, despite the fact that escape to the unconditioned stimulus invariably occurred.

When comparing these results in cats and rats one may speculate that all three structures studied are somehow involved in conditioned avoidance, but that the different components of this avoidance reaction bear different relationships to different structures. Septal lesions involving the fornix seem to wipe out the recollection of previously acquired conditioned avoidance but do not impair relearning. Since the critical structure in this syndrome seems to be the fornix rather than the septum itself, this fact would lend support to theories which claim that the hippocampus is involved in memory mechanisms. Amygdaloid lesions seem to impair acquisition of the response, possibly by reducing the motivational aspect of fear and anxiety as pointed out by Brady *et al.* (15), though, finally, conditioned avoidance will be acquired. It may be worth mentioning here that, according to Weiskrantz & Pribram (175), rhinencephalic lesions in monkeys not only impair the acquisition, but also quite strongly impair the retention of the conditioned avoidance response. On this point their results are at variance with those reported by Brady *et al.* (15). The case of orbitofrontal lesions in Brady's animals is still difficult to understand. These lesions apparently do not reduce the emotional response, and one would therefore suspect that they would not influence the motivational facilitation of retention or acquisition, yet avoidance does not occur for some as yet unknown reason.

An interesting contribution to the problem of the motivational forces

in behavior and their dependence upon rhinencephalic mechanisms is the experimental study by Olds & Milner (117) pointing out that stimulation of the septum, the cingulate gyrus, the mammillothalamic tract, and certain parts of the tegmentum in rats apparently produces effects which are experienced by the animal as a reward. The technique consisted of evaluating the time an animal would spend pressing down a bar which would close the switch of a circuit which stimulates the animal's own brain through chronically implanted electrodes. Stimuli delivered to structures presumably producing pain, e.g., stimulation of the medial lemniscus, induced the animal to avoid pressing down the bar. With stimulations applied to the aforementioned rhinencephalic and subcortical structures the animals would, however, seek to press down the bar as often as possible, thus obviously acting as if stimulation would produce a rewarding experience. The authors have good reasons to believe that this behavior indicates a strong pursuit of a positive rewarding stimulus produced by electrical stimulation. Since this is the first experimental series where the electrical stimulus produced a behavior suggesting the elicitation of a pleasant experience rather than the concomitants of unpleasant emotions like fear and rage, these observations by Olds & Milner have to be considered as a very important step forward in the investigation of the problem of brain mechanisms and emotions. [See also Brady (16), Delgado, Roberts & Miller (41), and Chiles (27).]

Bard & Mountcastle's (10) classical investigation on rage mechanisms received new attention by Rothfield & Harman (143), who repeated part of the original experiments confirming the original results that complete bilateral neocortical removal increases the rage threshold. However, they cast some doubt upon the importance of the amygdala in maintaining this placid state since in their experiments transformation of such a placid animal into a savage beast occurred after section of the fornix, while the amygdala was left intact. Section of the fornix in cases with only subtotal removal of the neocortex was without effect. It seems to them, therefore, that it is the hippocampus, rather than the amygdala, which acts as an inhibitor of subcortical rage mechanisms. Contradictory evidence to this, however, is furnished by stimulation studies reported by Delgado (40), showing that the outward expression of emotional reactions to noxious stimulations can be reproduced by stimulation of the posterior part of the hippocampus and of the crus of the fornix. This would indicate that the hippocampus activates, rather than inhibits, subcortical rage mechanisms, especially since in the same study the author shows that similar responses are obtained when stimulating the lateral part of the tegmentum, the central gray matter around the Sylvian aqueduct, and the posteroventral nucleus of the thalamus. In another experimental study Delgado, Roberts & Miller (41) have demonstrated that unpleasant emotional experiences (presumably fear) as elicited by electrical stimulation of the hippocampal gyrus can motivate learning just as well as can fear produced by painful electrical shocks applied to the skin, as used in classical conditioning experiments.

The role of the rhinencephalon in integration of emotional behavior is

further illustrated by clinical observations in man. An interesting contribution to this problem is a recent clinical study by Macrae (101), who comes to the conclusion that fear as a true epileptic aura has its origin in the uncus region of the temporal lobe. Additional evidence for this can be found in the recent monograph by Penfield & Jasper (123). As in animals, the emotion of fear in man is closely related to that of rage and often merges into it. It is therefore not surprising that a violent outburst of rage, associated with amnesia, sometimes occurs as an ictal epileptic phenomenon in focal discharge originating in deep temporal structures as reported by Gastaut *et al.* (56). These paroxysmal outbursts of rage are reminiscent of those seen in rabies; this is interesting in view of the fact that this disease mainly involves the hippocampus. In a recent study Gastaut & Miletto (57), moreover, point out that the paroxysmal phenomena of rabies in humans and animals are similar to those seen to occur in response to rhinencephalic stimulations, whereas the intercritical symptomatology of this disease bears some resemblance to changes seen in animals with rhinencephalic lesions.

*Social behavior.*—The integration of social behavior of an individual animal within his group was also found to be dependent upon rhinencephalic function. A study on social behavior in amygdalectomized monkeys by Rosvold, Mirsky & Pribram (141) reveals the effect of rhinencephalic lesions on interaction between individuals within a social group. While bilaterally amygdalectomized monkeys may appear more "aggressive" in an individual cage situation because of their lack of fear, this fearlessness is of no avail to them in the group cage situation since it does not correlate with increased outward aggressiveness. On the contrary, previously aggressive animals which before operation played a dominant role in the monkey colony as a result of their reckless aggressiveness slide all the way down the hierarchic scale after bilateral removal of the amygdala and become submissive and passive in the face of constant abuse and provocation, not even retaliating when openly attacked. A clinical report by Terzian & Ore (163) describing a Klüver-Bucy syndrome produced in man after bilateral temporal lobectomy somewhat duplicates these findings. There was complete disappearance of aggressiveness in a patient whose uncontrollable aggressiveness had been the main justification for this radical procedure. This diminution of aggressiveness after bilateral amygdaloid lesions in man is also reported by Sawa *et al.* (145). Their patients were all schizophrenics undergoing bilateral amygdalectomy for the purpose of reducing their aggressiveness. Gastaut *et al.* (56) moreover, point out that discharging lesions in the rhinencephalic portion of the temporal lobe as seen in psychomotor epilepsy apparently produce the opposite effects to those seen with lesions, i.e., a lowered rage threshold evidenced by the fact that these patients may show violent outbursts of temper for no adequate reasons. In contradistinction to true ictal phenomena of rage, these outbursts are always remembered and often deplored by the patients.

*Sexual behavior.*—It has already been mentioned that marked hypersexuality is produced by bilateral lesions of the temporal portion of the

rhinencephalon (Schreiner & Kling, 149, 150, 151). Clinical observations by Sawa *et al.* (145) and by Terzian & Ore (163) have shown that similar lesions in man, consisting mainly of bilateral amygdaloid lesions, may also be followed by some release of sexual drive. Neither report, however, gives any clear evidence for an increase in normal heterosexual tendencies, but both rather suggest an increase in sexual manifestations of a more immature type, either of an autoerotic character, as in Terzian & Ore's case, or in the form of an increased readiness to give verbal expression to sexual thoughts, as in the cases reported by Sawa. It is interesting in this respect to mention that, according to Gastaut & Collomb (55), irritative lesions of the rhinencephalic portion of the temporal lobe produce the opposite effect, that is, a decrease in sexual impulse. Their conclusions are based on clinical investigations demonstrating that in two-thirds of the patients suffering from psychomotor epilepsy with the discharging lesions presumably located within the rhinencephalic structures of the temporal lobe, this hyposexuality is found. Moreover, three patients who had no seizures, but suffered from loss of libido, had EEG evidence for a discharging lesion deep within the temporal lobe. The diminution of sexual drive in these patients, whether epileptic or not, was not associated with any signs of hypogonadism and, therefore, suggests that sexual behavioral mechanisms other than those primarily linked with endocrine functions may be dependent upon rhinencephalic activities. In view of these clinical observations, the conclusions drawn from Schreiner & Kling's experiments (151) about the probable endocrine origin of hypersexuality in bilaterally amygdalectomized animals may have to be reconsidered. The cause for these behavioral disturbances may not be endocrine at all, but rather neural. The integrity of endocrine mechanisms then would have to be considered as the necessary "biochemical" prerequisite enabling the brain to integrate sexual behavioral patterns. This would still be compatible with the fact that hypersexuality induced by rhinencephalic injury disappears after castration as reported by Schreiner & Kling (151).

The involvement of the rhinencephalon in sexual behavior is further substantiated by the analysis of the pathophysiology of rabies as studied by Gastaut & Miletto (57). These authors provide ample evidence from human and especially veterinary medicine that this disease, affecting primarily the hippocampus, is accompanied by disturbances of sexual behavior.

*Conclusions.*—These behavioral studies in animals and man demonstrate that the rhinencephalon must play an important part in the integration of complex forms of behavior. Most of the studies suggest that the motivational forces of behavior, as represented by emotions, are somehow dependent upon these structures; the extent of this dependence remains uncertain, and the numerous contradictions which still stubbornly remain irreconcilable with each other preclude at the present time the formulation of a coherent theory describing the role played by rhinencephalic function in the integration of emotional behavior. In any case, the wide range of activities, somatic, autonomic, endocrine, electrocorticographic, and behavioral, known to be influenced by rhinencephalic function would be compatible with the theory

that the rhinencephalon plays a major role in the elaboration of emotional behavior. That this system may participate in other complex functions of the brain as well is shown by effects on learning and memory to be cited in the following section.

## MEMORY MECHANISMS

There is now clear evidence that the rhinencephalic structures on the mesial aspect of the temporal lobes are intimately and critically concerned in human memory function. One source of data has been the work on brain stimulation in conscious epileptic patients where attention has lately been drawn to the role of the periamygdaloid region in the genesis of automatism and its attendant amnesic phenomena (46); such findings, though difficult to interpret, are of course immensely challenging for any theory of memory. However, there is also more direct evidence pointing to the importance of the mesial temporal region and, in particular, the hippocampal complex for normal processes of retention: we now know that destruction of these focal areas may cause severe and persistent memory loss, while leaving other aspects of intelligence essentially intact (108, 152).

Penfield has long insisted on the importance of the temporal lobes in the preservation of past experience (121). In a recent review of his stimulation studies (122) he has presented fresh case material illustrating the fact that electrical stimulation of the exposed temporal cortex under local anesthesia may cause patients with temporal-lobe epilepsy to have complex hallucinatory experiences which they accept, perhaps because of the accompanying feeling of familiarity, as authentic episodes from their past life. Such responses to stimulation occur in only about 25 per cent of temporal lobe cases; they arise chiefly from the superior and lateral surfaces of the temporal lobe, and occasionally from the adjoining part of the parietal lobe, but never from other parts of the cerebral cortex. Penfield attributes these phenomena to the reactivation of neuronal circuits involved in the preservation of past experience, but he does not claim that these neuronal circuits necessarily have their locus in the temporal neocortex.

The phenomena of temporal-lobe automatism also raise problems as to the physiological mechanism of retention. Thus Feindel & Penfield (46) report that 78 per cent of patients undergoing partial temporal lobectomy for the relief of seizures exhibit automatism either at the outset of the attack or after preliminary "psychical," sensory, or brief motor phenomena. It is characteristic of such attacks that they are followed by retrograde amnesia for the period of automatic behavior and sometimes even for a postictal period during which the patient is behaving normally. The attacks themselves are usually accompanied by spontaneous epileptic discharge in the mesial inferior temporal region, and in such cases electrical stimulation of this periamygdaloid area may elicit automatism and subsequent amnesia. The authors believe that for this to occur the discharge must spread to subcortical structures.

Direct proof of the importance of the mesial temporal region for normal

memory is provided by reports of memory loss after bilateral destruction of the hippocampal zone. As early as 1952 Glees & Griffith (61) described a case of bilateral destruction of the hippocampus and hippocampal gyrus in which gross memory impairment was an early and prominent symptom; they went on to suggest that in the adult the hippocampal formation is necessary for the maintenance of normal memory function. Striking support for this view comes from Scoville (152), who observed a grave loss of recent memory in one epileptic and one psychotic patient after bilateral surgical removal of the uncus, hippocampus, and hippocampal gyrus to a distance of roughly 8 cm. posterior to the tips of the temporal lobes. In his experience removal of the uncus and amygdala alone caused no memory loss.

More detail as to the nature of the deficit seen after hippocampal destruction is provided by Milner & Penfield's description (108) of memory loss in two cases of unilateral temporal lobectomy in the dominant hemisphere. In one case the removal involved two operations, five years apart, and the memory loss followed the second operation, at which time the uncus, hippocampus, and hippocampal gyrus alone were excised. Since in their experience generalized memory impairment did not follow unilateral temporal lobectomy, they concluded that in these two cases the hippocampal zone of the opposite side had been rendered largely incapable of function by birth compression, so that when the functioning but epileptogenic hippocampal zone on the left was removed the patients were functionally deprived of that zone on both sides. Psychological studies of these patients before and after the operation which produced memory loss showed the defect to be remarkably selective in that there was no change in intelligence quotient nor any defect of attention or concentration. Both men have continued to earn their living; they retain their old skills and their early memories, although there is some retrograde amnesia. On the other hand, they appear to retain little if anything of ordinary daily events, and the loss affects recognition as well as recall. Further analysis suggests that the essential deficit is the inability to recall an object or event once the attention has been switched to some other topic. If undistracted these same patients can retain an unfamiliar word-association or a short series of digits for many minutes, although they may invent elaborate mnemonic devices to help bridge the gap. Psychological study of Scoville's patients with memory loss has shown a similar pattern.

These observations suggest that the normal consolidation process by which experiences are retained beyond the period of primary attention depends upon the integrity of the hippocampal system, but the basic mechanism is far from clear. It is tempting to try to relate these findings to the various clinical studies claiming that memory defect is particularly likely to result from lesions of the third ventricle in the neighborhood of the mammillary bodies. This view, first put forward by Gamper (51) with reference to Korsakoff psychosis, has been hotly debated in the literature and some of the work cited in support of it (23) has not stood the test of later

statistical analysis (105). However, the question has recently been reopened in a more systematic manner by Williams & Pennybacker (177), who report the results of careful psychological testing (176) of 180 patients with verified intracranial lesions. Briefly, they found that amnesic disturbances were rare in neocortical lesions but frequent in lesions involving deep midline structures, particularly in those encroaching on the floor of the third ventricle. These results were not an artefact of age difference or of increased intracranial pressure. It is noteworthy that lesions in this region were rarely accompanied by gross disturbances of consciousness or intellect, but caused a selective impairment of memory. Moreover, the attention span of these amnesic patients, as measured by the immediate repetition of digits or single sentences, was completely well maintained. The parallel with the effects of hippocampal lesions as described above is, of course, immediate and striking.

In view of the known connections between hippocampus and mammillary bodies and their particular orderliness and prominence in primates (158) it is reasonable to suppose that damage to either of these structures in man interferes with a common system. The data reviewed here make it appear likely that this system is crucially concerned with the retention of current experience and not with emotionality, as formerly claimed (119). Moreover, this interpretation would accord better with the behavioral silence and physiological sensitivity of the hippocampus, which has occasioned much comment (130). Against the hypothesis perhaps is the apparent lack of gross memory defect after bilateral section of the fornix [Dott (42); Garcia Bengochea *et al.* (52)].

*Animal studies.*—It is regrettable that there should be so few animal experiments bearing on this general topic. The evidence so far available is both scanty and equivocal. On the positive side Rosner (140) found that in the rat cortical lesions which encroached on the rhinencephalon had a far more detrimental effect on the retention of a complex maze habit than did lesions of comparable size restricted to the neocortex. More specific to the hippocampus is Mishkin's negative finding (109) that, in the monkey, bilateral hippocampectomy (sparing however the hippocampal gyrus) caused little or no impairment in the retention of preoperatively acquired visual discrimination habits, a finding which closely parallels Allen's earlier study of differential olfactory conditioning in the dog (4). However, these negative results are not necessarily at variance with the human data, since the tasks used had been learned preoperatively and the amnesic human patients retained their preoperative skills. Furthermore, Mishkin used the massed trials training technique, the same problem being presented repeatedly with only a few seconds interval between successive discriminations, and we have seen that in the human cases the memory loss only showed up clearly when a distraction was interposed between stimulus presentation and recall. It is to be hoped that further animal studies of hippocampal function will use spaced trials and unfamiliar problems.

Finally, we must consider what bearing if any the changed reaction to

aversive stimuli (141, 174) and the variable effects on conditioned avoidance behavior (15, 174) seen after bilateral amygdalectomy in monkeys and cats may have on the broader problem of memory loss. These postoperative changes may seem at first sight to correspond to the rapid forgetting exhibited by the human patient with bilateral hippocampal lesions. But in man memory loss apparently does not follow amygdalectomy alone (152), nor is such loss specific to emotionally-toned stimuli. This means that, to be analogous, the deficit in animals would also have to show itself on a variety of learning tasks and not merely on those, such as conditioned avoidance, which use fear as a motivating agent. Such a general learning deficit has still to be demonstrated after a rhinencephalic lesion in the monkey.

## LOCALIZATION OF FUNCTION IN NEOCORTEX

*Animal work.*—Primate neocortical function has been discussed at length elsewhere (31, 70, 107, 164). This review will therefore confine itself to the most recent studies. These have been largely concerned with defining further the deficits caused by bilateral frontal, temporal, parietal, or occipital ablations in the monkey, making maximum use of experimental designs which permit the double dissociation of the effects of brain injury. If lesion I produces a deficit on Test A but none on Test B, whereas lesion II does the reverse, this means that the two areas destroyed were not functionally equivalent. In this way it was established that bilateral frontal lobe lesions cause deficits in delayed response and delayed alternation but do not affect visual discrimination, whereas the reverse is true for temporal lobe lesions, which cause deficits in visual discrimination but only rarely affect delayed response (31). We now know that damage to the inferior temporal convexity suffices to produce this visual deficit and that the areas implicated include the middle temporal, inferior temporal, and fusiform gyri (109, 110, 112).

This deficit is modality-specific (129, 178). Wilson (178) has shown that inferior temporal operates can carry out somesthetic pattern and size discriminations comparable in difficulty to the visual pattern discriminations on which they are severely impaired. This study provides an example of double dissociation, since Wilson also found that parietopreoccipital lesions caused persistent deficit in somesthetic discrimination but none in visual. Similarly, Weiskrantz & Mishkin,[5] working in the largely unexplored field of auditory discrimination learning, found that the inferior temporal operates performed as well as normal monkeys on a noise-versus-tone discrimination problem, although both frontal and posterior-temporal lesions produced impairment on this auditory problem while leaving visual discrimination intact.

Mishkin & Hall (111) have shown that inferior temporal operates have an increased difference threshold for visual size. On a graded series of size discrimination problems impairment was seen only on the more difficult items. Again, Chow (30) found that pattern and object discrimination learning sets

[5] Personal communication.

were differentially affected by temporal neocortical ablation, the former being lost and the latter retained. Such selective effects suggest a complex perceptual disturbance rather than a general loss of comprehension of the testing situation such as Lashley has proposed (28, 92, 93).

The various temporal lobe studies are consistent in showing a greater deficit in new visual learning than in the retention of old visual habits, though both are affected (31, 112). The degree of impairment is also positively correlated with the difficulty of the task for the intact animal (30, 109). This difficulty depends not only on the physical characteristics of the discriminanda (111) but also on the conditions of testing (131).

In an attempt to achieve a more precise definition of this complex deficit, Wilson & Mishkin (179) compared the effects of inferior temporal and lateral striate lesions upon a variety of visual tasks. Although the results implicated both areas in visual function, the deficits differed in the two groups. The striate animals showed maximum impairment on patterned string tasks, on which the temporal operates did not differ significantly from normal controls, whereas the temporal animals showed most deficit on learning set and pattern discrimination. Both groups showed a deficit on size discrimination, the striate animals being the more severely affected. However, the difference between the striate and the temporal lobe deficits cannot be neatly reduced to the difference between visual perception and visual learning. The temporal operates can learn a visual object alternation task at the normal rate, and they do have a deficit in visual size discrimination. Chow has attributed the temporal lobe deficit to lowered visual attentiveness (30), Mishkin to a reduction in the acquired distinctiveness of visual cues (110). The visual disturbance seen in human patients with epileptogenic lesions of the inferior and mesial temporal cortex would fit this latter description very well (107).

The next problem is to determine how the visual functions of the inferior temporal cortex are mediated. The observations of Sperry and his co-workers (160) that extensive subpial slicing and tantalum wire implantations in the visual cortex of the cat has no major effect on visual pattern discrimination argues against the importance of transcortical pathways, as also do many earlier studies (107). But Chow (29) has also reported negative findings from pulvinar lesions, though the fact that these were incomplete makes it premature to deny the importance of this thalamic nucleus in visual discrimination: the alternative would be a high degree of equipotentiality at the thalamic level. A somewhat different emphasis is given by Pribram (128) who postulates a downstream modulating effect of the temporal neocortex on visual function, possibly via the superior colliculus. Against this is the fact that Sperry's cats could do pattern discrimination despite extensive damage to the superior colliculi. This problem has still to be resolved.

We turn now to the frontal lobes, where a consistent finding has been the failure of frontal operates to master traditional delayed response and delayed alternation problems, in both of which a temporal gap intervenes between

cue presentation and response. The cortical area critically implicated is limited to the lateral surface anterior to the arcuate sulcus (11, 120, 133).

In a series of ingenious experiments Mishkin & Pribram (114) have shown that frontal operates can in fact perform within normal limits in a delayed response situation, provided that the traditional spatial pre-delay cues are replaced by nonspatial ones. Despite this, the crucial variable cannot be spatiality as such, since the frontal animals cannot learn a delayed object-alternation task in which they have to choose alternately one of two dissimilar objects irrespective of position (132). On the basis of these and other related findings Pribram & Mishkin conclude that the frontal operates' success on some variations of delayed response (114) and delayed alternation (113) depends on the pre-delay cue's having acquired differential distinctiveness through experience; without this the time interval cannot be bridged.

There remain two studies which demonstrate increased perseverative tendencies after frontal excisions in the rat (13, 102). Since neither study included an additional control group with posterior lesions we cannot tell whether the deficit is specific to the frontal cortex.

*Human studies.*—Space does not permit any detailed appraisal of the vast clinical literature; fortunately such reviews are available elsewhere (37, 84, 164). Instead, topics will be chosen to illustrate current research trends.

One general finding has been the inferiority of brain-damaged groups as a whole, when compared with normal control subjects in the solution of complex tasks (124, 154, 165, 166). Moreover, the posterior lesions tend to cause more impairment than anterior ones (154, 165) even on some tests which have been claimed to be peculiarly dependent on the integrity of the frontal lobes (69). However, the opposite position still has its defenders (69).

When we turn to more specific issues, we find a contrast in emphasis between animal and human studies of the posterior cortex. Whereas the former have dealt primarily with modality-specific deficits, the latter have drawn attention to disorders which apparently transcend the limits of one sense modality. As part of a comprehensive study of the long term effects of penetrating head injury Semmes *et al.* (155) devised an objective test of spatial orientation, which had the merit of yielding comparable scores for visually- and tactually-guided behavior. The test required subjects to walk along routes represented by maps, some of which were presented visually and others tactually. As might be expected from the clinical literature (37), the subjects with parietal lobe injury were significantly more impaired than were subjects with damage to other parts of the brain, and indeed the latter did not differ significantly from a control group with peripheral nerve injury. Of more interest is the fact that positive correlations were obtained between the visual and the tactual tests. The authors conclude that disorders of space perception which persist beyond the early posttraumatic phase are not specific to one sense modality.

These results are not at variance with animal studies. They merely show that whether one emphasizes modality-specific or transmodal defects after circumscribed brain injury will depend very much on the behavioral meas-

ures used. Orientation in space does not appear to be modality-specific for man or monkey. Wilson's (178) animals with posterior parietal lesions showed gross errors of spatial orientation, though only in the light, and she would like to attribute this to a changed relationship between visual and somesthetic systems. (It will be recalled that her parietal operates had a deficit in somesthetic discrimination, but none in visual.) Again, Orbach (118) found that bilateral occipital lesions, almost certainly involving prestriate as well as striate cortex, seriously interfered with a locomotor maze habit in blind monkeys, although the operation had no effect on a stylus maze habit or a variety of somesthetic discrimination problems. It should be pointed out that Orbach's monkeys had originally learned the locomotor maze with vision intact and had then been retrained to criterion after peripheral blinding. The occipital operation caused further impairment in these blind, maze-proficient animals, but whether it would have done so without the original visual training is unknown. Further investigation of these apparent intermodal effects would be of interest, especially as there is already some evidence that in man intermodal transfer may be impaired in different ways by brain injury, depending on where the lesion is (154, 165).

The long-term effects of brain injury are apt to be surprisingly mild; nevertheless they may still be detected if sufficiently refined quantitative measures are used. This has been illustrated recently in a series of elegant studies of somesthetic function by Weinstein (171, 172, 173). He found that men who had sustained penetrating head wounds years earlier still showed deficits in weight and size discrimination in the hand contralateral to the injury, the former appearing regardless of the presence or absence of primary sensory impairment as measured by two-point discrimination and tactile localization. He also demonstrated that the group with unilateral parietal lobe injury had an increase in the normal tendency to underestimate the first of two equal weights consecutively applied to the same hand ("negative time error"), and that this was true for both hands. Weinstein stresses the fact that the weight discrimination deficit only appeared with bilateral simultaneous stimulation, showing the importance of the testing procedure as well as the physical characteristics of the stimuli, a point which has been made earlier for animal studies (131).

No review of localization studies in man can properly neglect the domain of language, and here there are new data on temporal lobe function. Both Milner & Penfield (108) and Meyer & Yates (106) have reported deficits in the recall of specifically verbal material in association with lesions of the dominant temporal lobe. This deficit is dissociable from both aphasia and general memory loss, and does not accompany similar lesions of the minor temporal lobe (106, 108) or of the frontal and parietal cortex of either hemisphere (108). Milner & Penfield find the deficit before operation in patients with epileptogenic lesions of the dominant temporal lobe, Meyer & Yates only as a sequel to partial temporal lobectomy; this difference appears to reflect differences in the measures used. It is interesting that a deficit so specific to learning and retention should be temporal in origin, in view of the

close relationship between the temporal neocortex and the underlying amygdala and hippocampus, damage to which results in a more general memory loss. It raises again the problem of the functional relationship between neocortex and allocortex in the temporal lobe already posed by Penfield's stimulation studies (122) and by seizure phenomena (144).

A further topic specific to man which has lately come in for critical reappraisal on a statistical basis is that of the relationship between handedness and cerebral dominance. It is now explicitly recognized that this question can only be evaluated on the basis of a large number of consecutive cases of unilateral brain injury. Studies which meet this requirement (62, 138) agree in finding a higher incidence of aphasia after left-sided than after right-sided lesions even in left-handed individuals, provided that cases of infantile brain injury with right hemiplegia are excluded. The popular assumption of a simple symmetrical relationship between handedness and language laterality is thus no longer tenable. However, the nature of cerebral organization in predominantly left-handed people is still open to dispute. Roberts' (138) figures on the incidence of aphasia in left-handers as a sequel to cortical excisions indicate a much smaller proportion of cases with right hemisphere involvement than do data collected from other sources. He attributes this discrepancy to the fact that in the older literature negative cases were not always reported and concludes from his own findings that language is normally represented in the left hemisphere. On the other hand, Goodglass & Quadfasel (62) in an admirable discussion of this difficult problem favor the view that many left-handers have some measure of bilateral language representation (77). They also point out that the problem of laterality (and hence of its anomalies) is not specific to language, since there is evidence that the two hemispheres contribute unequally and perhaps in qualitatively different ways to various perceptual skills (107).

One recurrent problem in the study of human brain function has been to assess how much behavioral impairment can result from an active disease process or an active epileptogenic area simply by virtue of its interference with the functioning of adjacent healthy tissue. It has been claimed that such impairment may be more severe than that seen after clean surgical removal of the damaged area and some recent clinical observations support this view (91, 168). The problem is now being investigated experimentally in monkeys, the method being to create discrete epileptogenic lesions by alumina cream implantation and then to study the effects of the resulting cerebral dysfunction upon previously established habits and new learning (32, 127). Clear-cut positive findings come from Morrell, Roberts & Jasper (115, 116), who investigated the effects of experimental alumina cream lesions on conditioned alpha responses to sound, touch, and changes in background illumination. They found that a unilateral epileptogenic lesion in a given sensory receiving area (visual, auditory, or tactual) caused drastic impairment of the conditioned response for that particular sensory system but did not affect conditioning in other modalities. Moreover, when the epileptogenic focus was excised the conditioned alpha response returned to normal (116).

This proves that the focal epileptogenic lesions had had a deleterious effect on the functioning of nearby cortex, although the influence did not spread to other sensory systems. Amygdala lesions, however, disturbed both visual and auditory conditioning, showing that a strategically placed epileptogenic lesion can have a widespread nociferous effect. Further studies with other types of learning situation are needed before we can assess the generality of these findings.

## HALLUCINOGENIC AND ADRENOLYTIC COMPOUNDS IN CERTAIN MENTAL DISORDERS

Although adequate treatment of this subject is beyond the scope of this review it is believed that recent work with such hallucinogenic compounds as lysergic acid diethylamide (LSD-25) in relation to certain breakdown products of epinephrine (adrenaline) [e.g., adrenochrome (76)] when taken together with remarkable therapeutic effect of the new adrenolytic agent, chlorpromazine [chloro-3 (dimethyl-amino-3-propyl)-10 phenothiazine] (38, 39, 94) and reserpine (serpasil) (148) is of such importance that it should be mentioned in current thinking about the neurophysiological basis of higher functions of the nervous system. This bears a close relation to the suggestion of Woolley & Shaw (180) that certain mental disorders may result from serotonin deficiency since a variety of antiserotonins induce mental disturbances mimicking certain features of those seen in schizophrenia.

Marrazzi & Hart (71, 103, 104) have recently pointed out the chemical similarity between mescaline, epinephrine, adrenochrome, serotonin, and lysergic acid diethylamide, (LSD-25), all of which seem to inhibit conduction in cortical synapses, serotonin being the most effective. In unanesthetized animals Purpura (134) found differential facilitation and depression in different synaptic systems in thalamus and cortex while Evarts & Marshall (43) found that LSD abolishes the post synaptic potential in the lateral geniculate inducing subnormal excitability. It is premature to speculate regarding the relationship of these observations to the mechanisms whereby psychotic states are produced by these drugs, but further work, correlating neurophysiology and psychological data in this manner may lead to a better understanding of the neurophysiological basis of certain mental disorders.

Chlorpromazine has been shown by Hiebel, Bonvallet & Dell (12, 74, 75) to produce a striking depression in excitability of the epinephrine-sensitive portion of the reticular activating system in basal diencephalon and upper midbrain, a result confirmed by Angeleri *et al.* (6) and Rothballer (142). This would be consistent with changes in the EEG following administration of this drug to patients (8, 35, 54, 156, 162, 167). Such results, when related to clinical observations, have led Lehmann (94) to the conclusion that disturbances in the reticular activating system may play an important role in certain aspects of mental disease so that reintegration of thought processes becomes possible following control of the disturbing influence of excessive activity of the brain stem activating system by chlorpromazine. A similar conclusion is reached by Terzian (162).

These studies also emphasize the importance of humoral agents in controlling the excitability and responses of the brain stem reticular system and associated cortical projection systems as emphasized by Bonvallet *et al.* (12) and by Rinaldi & Himwich (135, 136, 137).

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# VISCERAL RECEPTORS[1,2]

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Among special reports and reviews dealing with afferent structures, we may refer to the contributions of Heymans (1, 2), Bykow (3), Dawes & Comroe (4), Green (5), Liljestrand (6), Rothlin & Konzett (7), Tchernigovski (8), Wyss (9), Aviado & Schmidt (10), Burn (11), and Granit (12).

## Cardiovascular Receptors

*Baroreceptors.*—It is generally admitted that nerve impulses originating from the cardioaortic barosensitive area reach the central nervous structures by way of the aortic depressor nerve, enclosed in the vagus trunk, or running as a separate nerve close to the vagus nerve. Several other pathways, which vary from one species to another, have been described, but without much convincing evidence (13, 14, 15).

Experiments recently performed on rats and rabbits by Andrew (16) showed that the fibres of some baroreceptors, located either on the anterior surface of the ascending aorta or on the aortic arch of the rat, course in the laryngeal recurrent nerve, join further the superior laryngeal nerve, and then reach the nodose ganglion of the vagus nerve. In rabbits, however, no positive evidence of aortic baroreceptors in the laryngeal loop could be obtained.

A consequence of Andrew's observations is that, in the rat at least, an unknown baroreceptor impulse pathway must be considered, and that, when it is desired to disconnect the cardioaortic reflexogenic area from the central nervous system, it would be necessary to interrupt also these afferent aortic fibres.

Investigations performed by Holmes (17) on the nervous pathways of the sinoaortic nerves in the cat have shown that a small number of baroreceptor afferent fibres originating in the carotid sinus are present in the aortic nerve; after section of the aortic nerve Holmes noted in the central end of the cut aortic nerve the presence of nervous impulses carried by baroreceptor afferent fibres originating in the carotid sinus.

Electroneurographic records on the right aortic nerve in cats (18) confirm earlier histological findings that the baroreceptor fibres originate from the junction of the subclavian and common carotid arteries and not from the aortic arch as on the left side. Moreover, two other baroreceptor areas, contributing to the right aortic nerve, have been described by Green

[1] The survey of literature pertaining to this review was concluded June 1, 1955. We do not claim to have covered the subject entirely and apologize for omitting perhaps some papers.

[2] The following abbreviations have been used in this chapter: TEA (tetraethylammonium); p.d.r. (pulmonary deflation receptors); HT (hydroxytryptamine).

(18). Both lie in the wall of the right common carotid artery and are proximal to the superior thyroid artery; this last site is of very practical interest, since in experiments a clip may unwittingly be placed on this baroreceptor area. These various areas have also been studied histologically by Boss & Green (19), and typical fibrillary nervous structures have been found in all baroreceptor areas.

According to Nakayama (20) electrical stimulation of various branches of the right aortic nerve, as well as distension of the brachiocephalic and subclavicular arteries, induce reflex responses in arterial pressure, heart rate, and respiration. It is concluded from these investigations that the superior depressor and the brachiocephalic branches of the right aortic nerve play a rôle in the reflex regulation of the vasomotor, cardioinhibitory, and respiratory centers. With regard to the anatomy of the aortic depressor nerve it should be remembered that, in 1924, Tello (21) described two main branches and pointed out that the left aortic nerve endings are located in the aortic arch, while the right branch innervates particularly the brachiocephalic and subclavicular arteries.

During operations on the thyroid gland, Akimov (22) observed that cardiocirculatory disturbances may arise by dividing or ligating the thyroid arteries. These disturbances are considered by the author as reflex responses to stimulation of vascular receptors. According to Akimov, histological preparations from the superior thyroid arteries reveal the existence of numerous receptor-like structures in the vascular walls. In the author's opinion the highly sensitive reflex area centred in the carotid sinus extends to the thyroid arteries and regulates the blood supply and the secretory activity of the thyroid gland.

Douglas & Schaumann (23) investigated the pressor and depressor effects of electrical stimulation of the central end of the aortic nerve in decerebrate cats with stimuli of different intensity and frequency. They observed that progressively increasing intensities cause first depressor, then pressor, and finally again depresssor blood pressure responses. Respiratory stimulant effects only appear as the depressor response changes to a pressor effect. The responses to aortic nerve stimulation are also dependent on the frequency of stimulation. The arterial pressure response can be made purely pressor by lowering the frequency of stimulation, and purely depressor by raising the frequency. The cat aortic nerve thus differs from somatic nerves in which low frequencies of stimulation favour depressor responses and high frequencies induce pressor effects.

The reflex regulation of normal systemic arterial pressure by the sino-aortic nerves has been sufficiently illustrated by many experimental and clinical observations. The inhibitory influence which these nerves exert on the normal vasomotor tone may be clearly demonstrated by experiments in which hypertension is found to develop when these buffer nerves are removed. Experiments of Heymans and co-workers (24, 25) have also demonstrated the fundamental rôle of the biologic condition and, particularly, of the

distensibility and resistance to stretch of the sinoaortic walls in the reflex adjustment and homeostasis of normal arterial pressure and, presumably, in the pathogenesis of arterial hypertension.

More recently, a new technique for producing hypertension in animals by altering the hemodynamics of the carotid sinus or of the brain or of both has been worked out by Wakerlin *et al.* (26). Dogs, subjected to bilateral constriction of the carotid sinus area by a plastic clamp, which causes a visible reduction in the pulse, were found to develop a persistent increase in systemic arterial pressure. The pathogenesis of this experimental hypertension is unknown. Modifications in brain hemodynamics with resulting stimulation of intracranial baroreceptors, or release into the plasma of pressor principles, called cerebrotonin and arteriohypertensin (27, 28, 29), have been considered as a possible cerebrogenic mechanism. According to Ead, Green & Neil (30), carotid sinus nerve impulses may decrease when pulsatile flow is changed to nonpulsatile flow, but hypertension only develops when the vagoaortic nerves have been eliminated. Finally, Wakerlin and co-workers emphasize the possibility—which we consider also as the most acceptable explanation—that impaired carotid sinus nerve function may be involved.

Some years ago Schafer (31) observed that an absolute polycythemia may occur in dogs made hypertensive by section of the four buffer nerves. It was suggested that hypertension with generalized vasospasm, accompanied by a local constriction of arterioles supplying the bone marrow and thus causing a decrease in marrow blood flow, stimulates erythropoiesis. Recently, Sisson *et al.* (32) repeated Schafer's experiments, using a slightly different method, and also examined in dogs the effects upon red cell volumes. The experiments performed by these authors give no indication that section of buffer nerves stimulates erythropoiesis.

An improved method for eliciting baroreceptor reflexes from the isolated carotid sinus has been described recently by de Burgh Daly (33). This method is similar to the procedure employed by Moissejeff (34). The isolated carotid sinus is subjected to a pulsatile pressure which can be varied independently of the mean pressure. With this technique active cardiovascular responses can be regularly obtained from the carotid sinus for periods up to 5 hr.

Experimental investigations of Alexander (35), who studied the participation of the venomotor system in pressor reflexes, have shown that hypertensive reflexes elicited by central vagal stimulation, carotid sinus hypotension, and hypercapnia or hypoxia, are found to produce reflex venoconstriction; intracarotid hypertension causes, however, reflex venodilatation, thus confirming previous observations of Fleisch and of Heymans in 1930 (15).

Experiments were performed by Kezdi (36) to establish the rôle of sympathetic innervation in the control of the state of contraction of the carotid sinus walls. Electrical stimulation of the superior cervical ganglia or sympathetic fibres supplying the carotid sinuses induces gradual hypotension and decrease of the carotid sinus pressor reflex. These experiments of Kezdi,

as well as those of Palme (37), who also investigated the function of the sympathetic innervation of the carotid sinuses, provide evidence that the superior cervical ganglion participates in the reflex regulation of arterial pressure. It is suggested that disturbances of this sympathetic innervation, resulting in relaxation and decreased pulsatile expansion of the carotid arteries, could contribute to the pathogenesis of hypertension. On the other hand, however, Floyd & Neil (38) obtained little evidence that the activity in the sympathetic innervation influences the responsiveness of the carotid sinus baroreceptors.

Jiménez Diaz and co-workers (39, 40, 41), using a cross-circulation technic in studying the humoral transmission of hypertensive effects of central vagal stimulation in dogs, suggest that a pressor substance, probably norepinephrine, is directly liberated from the arterial walls and not from the brain or from any gland. It is concluded that this endocrine function of the vascular walls may have an important part in circulation homeostasis.

The effects of artificially-induced hypothermia on the carotid sinus pressor reflexes in anaesthetized dogs have been studied by Dubecz and co-workers (42, 43). At 25°C. the hypertensive responses to carotid occlusion are moderately reduced; the reflex pressor responses still decrease during the rewarming period. Restoration of normal arterial pressure regulation is very slow and generally requires a few hours.

Nashat & Neil (44) also examined in anaesthetized cats the effects of hypothermia on baro- and chemoreceptor activities of the carotid sinus and compared the reflex responses at 37°C., 26°C., and again, after rewarming, at 37°C. Reflex changes of respiration and systemic arterial pressure of baroreceptor origin, elicited by raising the pressure in one isolated carotid, are invariably present at 26°C., but slightly delayed and slower than at 37°C. Reflex hyperpnea and hypertension of chemoreceptor origin, elicited by intracarotid injection of sodium cyanide, are still present at 26°C., but of less intensity than at 37°C.; systemic responses to anoxia are however very weak at 26°C. Chemoreceptor action potentials were also recorded and elicited by inhalation of 5 percent $O_2$ in $N_2$; the inspired gas mixture still causes at 26°C. a marked increase in action potentials; the onset of response, however, is delayed compared with that recorded initially and finally at 37°C.

Osuga (45) studied the influence of epinephrine and some other drugs on the afferent impulses from the carotid sinus nerves in cats, and suggests, without objective data, that the increase of action potentials induced by epinephrine results from stimulation of chemoreceptors.

According to Hazard and co-workers (46), drugs with local anaesthetic properties, when injected into the thyroid artery of dogs, produce a marked transient rise of systemic arterial pressure which is of reflex origin and is attributable to local inactivation of the carotid sinus nerve endings. Similar hypertensive effects were already observed by Hering (47), when anaesthetic drugs were applied locally to the carotid sinus area. Hazard and co-workers conclude from their observations that this reflex hypertensive response could

perhaps be used as a valuable qualitative test for drugs presumed to have some anaesthetic properties.

Robertson, Swan & Whitteridge (48) studied the mechanism of the hypotension which occurs when animals suddenly inhale volatile anaesthetics. From experiments performed on cats, it may be concluded that, in appropriate doses, ether, chloroform, and trichorethylene sensitize the carotid sinus and aortic baroreceptors; this increase in sensitivity contributes largely to the cardiovascular changes during anaesthesia. High concentrations of anaesthetics, however, abolish the reflex responses. According to Whitteridge & Bülbring (49), volatile anaesthetics also sensitize pulmonary stretch receptors.

Experimental investigations of Martini & Calliauw (50), on the cardiovascular effects of protoveratrine in anaesthetized dogs, have shown that this drug, injected intravenously into the coronary or pulmonary arteries, or locally applied to the carotid sinuses, provokes a bradycardia which is of reflex origin and results from stimulation of receptors located in the areas of the coronary and common carotid arteries. They also found that the hypotensive effects of protoveratrine are essentially reflex and mainly attributable to stimulation of the carotid sinus baroreceptors. The hypertensive responses to carotid occlusion may be markedly decreased or suppressed by protoveratrine, either injected intravenously, or locally applied to the carotid sinus area. According to Abreu *et al.* (51), protoveratrine lowers the systemic arterial pressure in the dog by acting upon receptors, the impulses from which travel to the vasomotor center via the vagi and the carotid sinus nerves. Observations with veratridine, in vagotomized dogs, showed that its hypotensive effects were not always abolished after denervation of the carotid sinuses, thus providing an indirect support for the existence of a central site of action of veratridine.

As a result of the finding that the nodose ganglion of the vagus nerve is involved in the emetic action of Veriloid,[3] Borison *et al.* (52, 53) thought it possible that this ganglion might also be concerned in the hypotensive effect of this drug. The results of their investigations in vagotomized cats indicate that the hypotensive action of Veriloid is primarily mediated via the carotid sinus baroreceptors; the nodose ganglion may also participate in this hypotension and be important in cats with hypoactive buffer reflexes. No evidence of a central depressant action could be obtained.

Fernandez & Cerletti (54), studying the hypotensive effects of protoveratrine in anaesthetized cats, observed that therapeutic intravenous doses of pure protoveratrine A (1.5 to 2.5 μg. per Kg.) produce a fall of systemic arterial pressure which depends on intact vagal and carotid sinus innervation. The rôle of the carotid sinus during the first phase of arterial pressure response to protoveratrine is characterized by a compensatory buffering action, thus reducing the initial hypotensive response. In the second phase, the carotid

[3] Veriloid: trade name of a mixture of ester alkaloids of *Veratrum viride*.

sinus contributes to the maintenance of the hypotension, because protoveratrine directly acts on the carotid sinus baroreceptors and still produces a hypotensive effect in vagotomized animals, an action which is reversed to a hypertensive effect by denervation of the carotid sinus. In previous experiments it was demonstrated by Cerletti *et al.* (55) and by Rothlin & Cerletti (56) that veratrum alkaloids directly stimulate both chemo- and baroreceptors; the hypotensive effect is mainly due to stimulation of the baroreceptors, whereas the chemoreceptors react with a respiratory response.

The mechanism of vasomotor action of various veratrum derivatives has also been studied in anaesthetized cats and dogs by Wang *et al.* (57). In vagotomized animals, hypotensive effects were observed when appropriate doses of Veriloid, protoveratrine, germitrine, neogermitrine, or germerine were administered either intravenously or locally on the carotid sinus walls. During the hypotensive reaction, the carotid sinus pressor reflex is decreased or eliminated, and the excitability of the medullary vasomotor center is increased. The hypotensive response is independent of the activity of the carotid body and therefore is interpreted as being attributable to increased activity of the carotid sinus baroreceptors. Nodose ganglionectomy does not affect the hypotensive effect. On the other hand, it was found that veratridine and veratramine markedly depress the vasomotor center and it is believed that in these cases the carotid sinus baroreceptors play only a secondary rôle in causing the hypotensive effect.

Experiments of Moran *et al.* (58, 59) have shown that andromedotoxin, an active principle from *Rhododendron maximum*, locally applied to the carotid sinus or intravenously injected in vagotomized dogs, produces a marked systemic hypotension; after carotid sinus denervation andromedotoxin becomes ineffective or causes a rise in arterial pressure. It is concluded that the hypotensive action of this drug is mainly of reflex origin and results from stimulation of the carotid sinus baroreceptors.

A comparative study of various veratrum-like drugs (60) also showed that protoveratrine, neogermitrine and andromedotoxin exert a stimulating action on the carotid sinus baroreceptors, while veratridine, after an initial transient excitatory effect, blocks these receptors.

We fully agree with Gruhzit's *et al.* (61) comments and conclusion that it would be erroneous to explain the mechanism of action of any one veratrum alkaloid or exact alkaloidal mixture of veratrum in terms of the observed effects of any other single veratrum alkaloid or unknown mixture of such.

Experiments of Heymans & van den Heuvel-Heymans (62) have shown that 5-hydroxytryptamine (HT) causes, in dogs, a respiratory stimulation mainly of central origin. Douglas & Toh (63) assert, however, that these hyperpneic responses to HT are primarily attributable to stimulation of afferents running in the carotid sinus and vagus nerves, and perhaps partly depend on a central site of action. The respiratory and cardiocirculatory effects of HT have also been studied in cats by several investigators, but

with diverging results (64, 65, 66). Ginzel & Kottegoda (64) observed that small amounts of HT injected into the carotid sinus or aortic body regions cause a fall of systemic arterial pressure and respiratory stimulation. Both of these effects are abolished after section of the sinus or vagus nerves respectively. The hypotensive effect and the hyperpneic response are thought to be due to baroreceptor and chemoreceptor excitation respectively. It was also found that, after denervation of the carotid sinus, larger doses of HT may again cause similar respiratory and hypotensive responses, presumably of central origin. Schneider & Yonkman (65) observed that intravenous injections of HT produce qualitatively and quantitatively different respiratory and cardiovascular effects in dogs, cats, and rabbits; the species differences in the cardiocirculatory and respiratory responses to HT are largely caused by reflexes originating in the heart and the lungs; pulmonary stretch receptors are activated in dogs and cats only.

Recently Kottegoda & Mott (66) reinvestigated in cats the cardiovascular and respiratory effects of HT regarding its site (or sites) and mode (or modes) of action. In an attempt to elucidate the different phenomena, a large number of experiments was performed, and HT, in varying doses, was injected intravenously or intra-arterially. These experiments have shown that HT produces reflex bradycardia and hypotension by stimulation of afferent vagal fibres. HT also causes a reflex apnea mediated by some afferent fibres in the vagus, and it seems rather unlikely that HT stimulates directly pulmonary stretch endings; changes of action potentials from pulmonary stretch fibres are supposed to be secondary to direct bronchoconstriction caused by HT.

According to Dasgupta & Werner (67), carotid sinus hypertensive reflexes are completely abolished after intracisternal injection of small doses of "Largactil"[4] in anaesthetized monkeys; pressor responses of reflex and central origin are also abolished by intravenous injections of appropriate amounts of Largactil in decorticate cats. The authors claim that, in certain circumstances, a very pronounced suppression of carotid sinus vasomotor reflexes of central origin may be provoked by Largactil.

Several experimental investigations of Heymans and co-workers (68 to 77) have demonstrated that drugs contracting the vascular walls of the carotid sinuses cause a stimulation of the baroreceptors. De Vleeschhouwer, Martini & Calliauw (78) also showed that vasoconstrictor drugs applied to the cardioaortic area exert a stimulating action on the aortic baroreceptors followed by a reflex fall of arterial pressure. Drugs relaxing the carotid sinus vascular walls greatly diminish the baroreceptor activity and thus induce a reflex rise of arterial pressure (79). These experimental facts demonstrate the fundamental rôle of the biologic condition and, particularly, of the distensibility and resistance to stretch of the sinoaortic walls in the reflex regulation and homeostasis of arterial pressure.

[4] Largactil: trade name for chlorpromazine [3-chloro-10 (3'-dimethyl-aminopropyl)-phenothiazine].

Martini & Rovati (80) confirmed these observations and showed that phentolamine methanesulfonate (Regitine), an imidazoline derivative with adrenolytic properties, when locally applied to the carotid sinus walls, induces a marked reflex systemic hypertension; it also suppresses or reverses the action of epinephrine and norepinephrine on the vascular walls of the carotid sinus and thus inverts the reflex action of these drugs on the systemic arterial pressure. According to Martini & Rovati, the rise of arterial pressure induced by application of Regitine (which does not exert a direct vasodilating action) on the carotid sinus should be ascribed to its capacity of reversing the constricting action of the epinephrine contained in the vascular walls. This hypothesis requires, however, more convincing evidence and could perhaps be accepted, if it could be proved that Regitine, in the given doses, does not relax smooth muscles and has no local anaesthetic effects.

Experiments performed on anaesthetized dogs by Halpern, du Bouchet & Neveu (81), have shown that intravenous injection of therapeutic doses (0.5 to 3 mg. per kg.) of hexamethonium markedly depresses the vasopressor reflexes of carotid sinus origin, while the hypertensive responses to epinephrine and electrical stimulation of the central end of the vagosympathetic nerve are considerably enhanced.

On the basis of some observations it has been shown that the carotid sinus and aortic buffer nerves not only exert a regulatory action upon the activity of the autonomic nervous system, but may also influence some somatic functions (15). Experiments of Pinotti & Granata (82), performed on anaesthetized dogs, have shown that stimulation of carotid sinus baroreceptors, caused by increasing the intracarotid pressure, considerably depresses the linguomandibular reflex. Pinotti & Granata (83) also observed that stimulation of the carotid sinus nerves inhibits the knee-jerk reflexes in normal and decerebrated dogs without altering direct muscular excitability. It is concluded that this inhibitory action follows a reflex pathway whose central connections are at a level below the mesencephalon and localized in the spinal cord. The authors also observed that the carotid sinus nerves inhibit the spontaneous movements and tetanic contractions which frequently occur in chloralosed animals; these effects are very marked and mostly appear before inhibition of spinal reflexes.

Experiments performed by Bonvallet and co-workers (84 to 89) on dogs and cats have shown that the baroreceptors of the carotid sinus area (and possibly of the aorta) may inhibit the tonus of the sympathetic centers and the electrical activity of the cortical centers, and this without variations of the arterial blood pressure or without changes of circulating epinephrine. They also showed that arterial hypertension, associated with increased concentration of circulating epinephrine, is able to inhibit the spinal motor activity; this is a reflex mechanism, since this inhibition depends upon discharges originating in the baroreceptors which activate the bulbar inhibitory portion of the reticular formation. Increase of circulating epinephrine may, however, produce a facilitation of the spinal motor activity mediated by the

part of the reticular formation localized in the anterior part of the brain stem; the posterior part of this system may also be activated by epinephrine if the baroreceptors are excluded. It follows from these experimental observations that epinephrine acts directly on certain reticular neurons; the stimulating action of epinephrine upon the reticulocortical and the reticulospinal systems considerably enhances the level of activity of the whole somatic system. A counter-regulation is exerted by the baroreceptors of the carotid sinus (and possibly of the aorta), acting by way of the inhibitory bulbar portion of the reticular formation, and moderates the output of the muscular system, thus coordinating its activity with the capacities of the circulatory system at a given time.

Dell & Bonvallet (86), studying the effects of hypercapnia and of hypoxia upon the reticular system in the spinal dog and cat, observed that carbon dioxide acting humorally directly stimulates the anterior part of the ascending reticular system; hypoxia also increases the activity of the reticular system, which is essentially reflex and mediated by the chemoreceptors of the carotid body; the direct central effects of hypoxia are however purely depressive.

Boxill & Brown (90) concluded from experiments on dogs that the baroreceptor reflexes, activated by increasing the intracarotid and intraaortic pressure, are unable to reduce the amount and the duration of the hypertensive effects of epinephrine.

In recent experiments, performed on dogs under barbital anaesthesia, and designed to test the postulate of baroreceptor impotence under more critical conditions, Brown & Hilton (91, 92) examined the cardiovascular responses to epinephrine and norepinephrine before and after denervation of the sinoaortic baroreceptors. On the basis of the obtained data, the authors claim that the baroreceptors are inefficient and incapable of moderating or reducing either the rise of arterial pressure or the duration of hypertension produced by intravenously injected epinephrine or norepinephrine; intact baroreceptors may even prolong the hypertensive response. It is finally concluded that, if the baroreceptors have any biological importance, it is probably more in the direction of protection against hypotension rather than against hypertension.

We cannot agree with these conclusions, and some of these data should be considered with circumspection. In the above experiments dogs were anaesthetized with barbital; this drug is known to depress considerably the baroreceptor reflexes and hence reflex regulation of the vasomotor system will be at least partially excluded (93). No after-control for adequacy of denervation of the baroreceptive areas seems to have been carried out. The authors presume that the baroreceptors more probably protect against hypotension rather than against hypertension. This assumption can hardly be reconciled with the physiological properties of the buffering baroreceptors which, in normal conditions, constantly moderate the activity of vasomotor centers. It should also be remembered that experiments of Heymans in 1929 (94) and

especially of Cuypers (95)—apparently ignored by Brown & Hilton—have clearly proved that the hypertensive and the hypotensive effects of epinephrine and acetylcholine, respectively, are compensatorily regulated and markedly moderated by the carotid sinus and cardioaortic baroreceptor mechanisms.

On the basis of experimental investigations performed on anaesthetized dogs, Gruhzit, Freyburger & Moe (96) conclude that the vasodilatation in skeletal muscles which accompanies the vasoconstriction in other organs, resulting from intravenous injection of epinephrine (or norepinephrine in somewhat larger doses) is of reflex origin. This reflex vasodilatation is not prevented or eliminated by section of the sinus, vagus, thoracic sympathetic, or phrenic nerves. The reflex is shown not to be primarily due to activation of the receptors of the Bezold reflex, or of chemoreceptors within the cardiac or pulmonary circulation, nor to alterations of mean pressure within the atria or the pulmonary vessels. The vasodilatation occurs with doses of epinephrine which fail to cause general hypertension. Section of the thoracic dorsal roots, however, completely abolish the reflex. The sensory elements are found to be located in the thoracic aorta and its branches and seem to be mechanoreceptors. The central connections and afferent pathways are still unknown. It should be remembered that experiments of Heymans *et al.* (97), Heymans, Bouckaert & Wierzuchowski (98), and of Heymans & Bouckaert (99), performed respectively in 1936, 1937, and 1938, have already shown that epinephrine may provoke, in sinoaortic denervated and in spinal dogs, a reflex vasodilatation in different vascular areas and organs. On the basis of their experimental findings it was concluded that some vascular areas possess reflex sensitivity to pressure variations, and it was pointed out that these vasomotor reflexes are chiefly spinal reflexes, the receptors being located in the area supplied by the celiac and superior mesenteric arteries; these spinal vasomotor reflexes may probably play a physiological rôle in the distribution of blood in the abdominal circulation and in the periphery.

*Chemoreceptors.*—During the last decade a large number of experiments has been carried out with regard to the mode of action of various drugs on chemoreceptors. The particular problem of transmission mechanism (or mechanisms) at chemoreceptors has been recently reviewed (100, 101, 102).

According to Liljestrand (100), there is little doubt that acetylcholine or a closely related agent plays an important rôle as a transmitter for certain receptors (e.g. chemoreceptors of the carotid body, taste receptors in the frog's tongue). His arguments are mainly based on detailed study of autonomic drugs involving the use of nicotine-like drugs, ganglion-blocking agents, and drugs preventing destruction of acetylcholine by inhibition of cholinesterases. But Liljestrand also writes,

> it must be admitted that as yet a release of acetylcholine from the active carotid body has not been definitely proved, and the possibility cannot be excluded that the transmitter substance consists of some unknown choline ester. . . .

On the other hand it is noteworthy that de Burgh Daly (101), basing

also his opinion on a critical analysis of arguments drawn from experiments on acetylcholine, acetylcholinesterases, nicotine, and hexamethonium, concludes that it is still doubtful whether acetylcholine plays a rôle as a transmitter for chemoreceptors.

In answer to the question of the existence of a chemical transmission at chemoreceptors, Douglas (102) states:

> It seems rather that a better hypothesis to explain the actions of acetylcholine and hexamethonium and related drugs is this: that the carotid body afferents in addition to being responsive to oxygen lack, possess a sensitivity to acetylcholine and similar drugs which is altogether independent of normal transmission mechanisms. The argument that acetylcholine is involved in chemosensory transmission appears to stand but poorly at present. . . .

The activity of carotid and aortic chemoreceptors may be markedly affected by the chemical changes of the arterial blood and by reductions of blood flow leading to local anoxaemia in these receptors. De Burgh Daly, Lambertsen & Schweitzer (103) investigated the volume of blood flow and the oxygen consumption of the carotid body in cats. These experiments suggest that the carotid body can be stimulated by intrinsic anoxaemia in the presence of a normal arterial $pO_2$.

Osuga (104), studying the influence of potassium and calcium chlorides on the action potentials of perfused carotid sinuses of cats, asserts that the increase of impulses caused by potassium chloride is evoked from the chemoreceptors. While potassium salts exert a stimulating action, calcium chloride has a blocking effect on the afferent impulses of the carotid sinus.

Experimental investigations of Landgren *et al.* (105) on the impulse activity in the carotid sinus nerve following intracarotid injections of sodium iodoacetate, histamine, *N*-benzyl-*N'*,*N'*-dimethyl-*N*-phenylethylenediamine (Lergitin), and some derivatives of purine and barbituric acid have shown that small doses of histamine do not affect chemoreceptor activity and that antihistaminic drugs do not abolish the chemoreceptive potentials elicited by oxygen lack; large amounts of the antihistaminic Lergitin suppress the chemoreceptive impulse activity, probably as the result of the local anaesthetic action of this drug. These observations show that histamine, which has been considered as a possible chemical transmitter in the carotid body, is not concerned with the stimulation and activity of the chemoreceptors. According to these results, the authors rather admit the hypothesis that acetylcholine acts as a transmitter substance in the chemoreceptors.

Since it has been suggested that bradycardia in icterus may be partly of reflex origin and attributable to actions of bile acids on sinoaortic receptors, experiments have been carried out by Erdmann & Rohr (106) in order to elucidate the effects of these acids on heart rate. Investigations in dogs have shown that the bile acids produce bradycardia by direct stimulation of the vagal cardioinhibitory center.

In continuation of their earlier investigations, Liljestrand & Zotterman (107) studied, in anaesthetized cats, the effects of close arterial injection of certain choline esters and of some strong anticholinesterases on the action

potentials from the carotid body chemoreceptors. The authors conclude from their experiments that the obtained results support the hypothesis that acetylcholine or related substances are involved in the initiation of the chemoreceptive discharge from the carotid body.

Experiments of Douglas (108) have shown that tetraethylpyrophosphate, a strong anticholinesterase, injected or perfused in the carotid body area, exerts no appreciable effect on the normal physiologic response to oxygen lack, although the response to injected acetylcholine is markedly increased.

Gollwitzer-Meier & Witzleb (109) investigated in cats the effects of some methonium derivatives on the activity of the carotid body chemoreceptors; small amounts of decamethonium, directly injected into the carotid artery, have no appreciable influence on the chemoreceptor potentials, whereas large doses may exert a secondary effect from respiratory paralysis; pentamethonium inhibits the action potentials even during anoxia or after preliminary injection of lobeline or acetylcholine.

More recently, Gollwitzer-Meier & Witzleb (110) also examined in cats the effects of some adrenolytic compounds on afferent elements of the autonomic and somatic nervous system. Dibenamine,[5] Hydergin,[6] tolazoline (Priscol), and phentolamine (Regitine), injected intra-arterially near the carotid body, produce a reflex transient hyperpnea and rise of arterial pressure of chemoreceptive origin; all drugs also stimulate the chemoreceptor action potentials, but chemoreceptive responses to anoxia remain normal; action potentials from carotid sinus baroreceptors are unaffected. Not only the chemoreceptive elements of the autonomic nervous system, but also the action potentials of afferent sensory paths (saphenous nerve) are found to be stimulated by the various adrenolytic drugs.

According to Heymans *et al.* (76), large doses of tetraethylammonium, hexamethonium, methantheline (Banthine), or Pendiomide, intravenously injected in dogs, do not affect significantly the respiratory responses to stimulation of carotid body chemoreceptors by acetylcholine, lobeline, or potassium cyanide; local application of these ganglion-blocking agents on the carotid bodies also does not noticeably affect the sensitivity of the chemoreceptors towards acetylcholine, lobeline, and potassium cyanide.

On the other hand, experiments of Dontas & Nickerson (111) have shown that tetraethylammonium, hexamethonium, and pendiomide depress chemoreceptor action potentials. Responses to acetylcholine or lobeline are delayed in onset and lowered, or completely abolished, following administration of the blocking drug through the carotid artery, while chemoreceptor responses to anoxia or to potassium chloride are unaffected, except by very high doses of blocking agents. In appropriate doses, quaternary, and, to a lesser degree, tertiary ganglion blocking compounds may increase chemoreceptor activity.

[5] Dibenamine is *N,N*-dibenzyl-β-chloroethylamine.

[6] Hydergine is a trade name of a mixture containing equal parts of the methanesulfonates of dihydroergocornine, dihydroergocristine, and dihydroergotkryptine.

According to Rothlin & Konzett (112) it is noteworthy that synaptotropic compounds, acting on autonomic ganglia, also exert effects on afferent structures which have no synapses, e.g. the carotid body chemoreceptors and the cardiac receptors which are responsible for the Bezold-Jarisch effect. Although it is not an absolute rule, these afferent structures may be stimulated by substances which stimulate the sympathetic ganglia and may be blocked by drugs which block sympathetic synaptic transmission.

According to Budde *et al.* (113), intracarotid injection of moderate to large amounts (1 to 20 mg.) of tetraethylammonium (TEA) in anaesthetized cats, stimulates the action potentials of the chemoreceptive carotid sinus afferents and induces a reflex hyperpnea; stimulating actions of acetylcholine or lobeline on the chemoreceptors are abolished, while the effects of hypoxia are scarcely affected by TEA. Larger doses of TEA may considerably reduce the action potentials. The authors also observed that "close arterial injection" of TEA (200 μg. to 20 mg.) considerably increases the action potentials from sensory nerve fibres (saphenous nerve); large amounts of TEA (200 mg.) inhibit these action potentials. It is supposed that the excitatory effect of TEA on action potentials is attributable to sensitization of the saphenous nerve to potassium ions.

In recent investigations on the influence of phenothiazine derivatives on autonomic nervous structures, Budde & Witzleb (114) observed that intracarotid injections of these drugs reduce the action potentials from carotid sinus chemo- and baroreceptors and also inhibit the responses to acetylcholine and lobeline. Large amounts of the drugs suppress completely the excitatory effects of hypoxia on the chemoreceptors and abolish the baroreceptive potentials, probably as the result of a local anaesthetic action of the injected drugs.

Several investigators, recording the afferent impulses from the cardiac vagus of the cat, have shown that cardiac stretch receptors are present in the atrial walls. Paintal (115) described two types of receptors: type B stretch receptors responding and adapting themselves to changes in atrial filling, and type A receptors stimulated by changes in the intra-atrial pressure. The specific functions of these intra-atrial receptors are not yet elucidated.

Recent experiments of Goto (116) have also shown that a sudden elevation in the perfusion pressure of the heart of toads causes a definite vasodilatation. It was proved that stretch receptors in the wall of the right atrium are, though not alone, responsible of this vasodilator reflex; the afferent fibres involved are contained in the cardiac vagal branches, while the efferent pathways of the reflex are at least in the splanchnic nerves and sciatic plexuses.

### Pulmonary Receptors

*Mechanoreceptors.*—The experimental investigations of Widdicombe (117, 118), carried out to localize the pulmonary receptors in the cat, are of particular interest. In a first group of experiments receptors in the trachea and bronchi were studied. Action potentials of afferent nerve fibres were

registered. Three types of tracheobronchial receptors, baroreceptive and chemoreceptive, were identified; mechanoreceptors were also detected in the mediastinum. The stretch receptors in the extrapulmonary bronchi and the trachea have many properties similar to the pulmonary stretch receptors; both are slowly-adapting and inhibit inspiratory activity.

On the other hand Widdicombe (119), registering phrenic nerve potentials after pulmonary inflation, observed three reflex responses: the Hering-Breuer inhibitory reflex, the excitatory response to large inflations (Head's paradoxical reflex), and a third reflex which is a short inhibition of inspiratory activity following a large inflation of the lungs and is distinct from the Hering-Breuer reflex. The three reflexes are differentiated by progressive cooling of the vagi.

In order to localize exactly the pulmonary stretch receptors, Widdicombe (120) recorded impulses from a single afferent fibre in the vagus. He observed that stripping the visceral pleura does not destroy the endings, thus showing that they are not pleural receptors; deep pressure even after pleural removal is still capable of stimulating the receptors. Drugs which produce bronchoconstriction cause an increased discharge from the receptors, while bronchodilatation is accompanied by a reduced discharge. It was also observed that the Hering-Breuer-reflex is more active after bronchoconstrictor drugs and less active after bronchodilator agents. It was finally concluded that many stretch receptors are localized in the intrapulmonary bronchi, although this site is not exclusive. The activity of these pulmonary stretch receptors appears to be related to bronchial tone.

According to Josenhans (121), electrical stimulation at different frequencies of the afferent lung vagus in rabbits, produces respiratory effects similar to those normally obtained from the pulmonary stretch receptors activated by different lung volumes. It is very likely that of the great number of sensory lung receptors only the stretch receptors seem to be important in physiological breathing. Josenhans (122) also compared the respiratory responses to stimulation of the afferent lung vagus with respiratory reactions caused by expiratory or inspiratory closure of the trachea. It was expected that low-frequency stimulations would have the same effects as expiratory trachea closure and vice versa, but it was found that electrical stimulation has never the same effect as expiratory tracheal occlusion.

Tachypnea may be produced experimentally by pulmonary emboli, congestion, and phosgene inhalation; double vagotomy abolishes this tachypnea, thus showing the rôle of reflex pathways in the vagi. Christie (123) suggested that this tachypnea is attributable to stimulation or sensitization of pulmonary stretch receptors. Bülbring & Whitteridge (124) could not observe an increased sensitivity of pulmonary stretch receptors during pulmonary congestion and concluded that other afferent vagal fibres are probably responsible for accelerating the respiration. Dawes *et al.* (125) showed that tachypnea could be produced by injecting guanidine derivatives into the right atrium but not into the left. Paintal (126) also studied the effects of

phenyl diguanide on pulmonary and cardiovascular receptors and found that this drug activates previously inactive vagal afferents of small conduction velocity. It was shown that most of these fibres come from the stomach and intestines (127). Further investigations of Paintal (128) revealed two vagal afferent fibres yielding a discharge of impulses with an unusually short injection-response time; these fibres probably come from receptors in the lungs.

In recent experiments, performed on anaesthetized cats, Paintal (129) endeavoured to determine the exact site and the particular characters of these receptors. It was pointed out firstly that the endings of certain vagal afferents are definitely located in the lungs, and, since they are specifically sensitized by lung deflation, they should be called pulmonary deflation receptors (p.d.r.). The site of these p.d.r. is different from that of the pulmonary stretch receptors, which have been shown to lie in the intrapulmonary bronchi [Widdicombe (120)]. The p.d.r. are probably situated near the alveoli. Just as the pulmonary stretch receptors signal the state of inflation of the lung, the p.d.r. probably serve to signal deflation. It also appeared that the p.d.r. are normally inactive; they are unaffected by anoxia, but sensitized by pulmonary congestion. The receptors are stimulated (in some conditions only sensitized) by injection of phenyl diguanide, starch, nicotine, and 5-hydroxytryptamine. It is suggested that the p.d.r. are primarily responsible for producing reflex tachypnea and may cause respiratory inhibition when strongly stimulated by drugs. The p.d.r. may also take part in the reflex bradycardia following injections of the above drugs. The receptors are not connected with the bronchial, but probably with the pulmonary circulation. Paintal finally states that the p.d.r. are quite different from Adrian's (130) deflation receptors which are perhaps slowly-adapting bronchial receptors (Widdicombe, 118).

Giulio & Mongini (131), studying proprioceptive respiratory reflexes in man, observed that a sudden increase or decrease of respiratory resistance is capable of eliciting a compensatory stimulation or inhibition of the corresponding respiratory phase. These compensatory responses, which are probably of reflex origin, never occur when the variations of respiratory resistance are slow and gradual.

In an interesting group of experiments, carried out on anaesthetized rabbits, Beck (132, 133) observed that inhalation of warm air is followed by a reflex increase of systemic arterial pressure. Evidence was obtained that the afferent pathway involved in this reflex hypertensive response is in the trigeminal nerve and the efferent pathway in the splanchnic nerves.

*Chemoreflexes.*—It is well known that some drugs, with veratrine-like properties, intravenously injected, produce apnea, bradycardia, and systemic hypotension. These have been called the "Bezold-Jarisch" effect and were attributed in part to a stimulation of sensory receptors in the heart, the afferent pathways running in the vagus nerves (von Bezold & Hirt, 134). On the basis of the observation that these depressor cardiac, vasomotor, and respiratory responses are reduced or abolished by vagotomy and thus are of

reflex origin, many investigators tried to elucidate the mechanisms involved in these reflex phenomena.

Dawes (135) proved that pulmonary receptors are also implied in the veratrinic depressor effects and showed that, whereas the reflex apnea is attributable to an action upon lung receptors, the reflex fall in pressure and heart rate results from an effect upon receptors in the heart and partly in the lungs. A critical analysis of the mechanisms responsible for the so-called Bezold-Jarisch reflex has been worked out by Dawes & Comroe (4). Three mechanisms at least must be postulated in order to explain the observed depressor actions: (*a*) the coronary chemoreflex, a reflex fall of arterial pressure and heart rate caused by injection of certain drugs into the coronary circulation (Bezold-Jarisch effect); (*b*) the pulmonary depressor chemoreflex, a reflex fall of pressure and heart rate elicited by an action on sensory receptors in the lungs; (*c*) the pulmonary respiratory chemoreflex, a transient apnea which is also attributable to an action on sensory lung receptors. In addition, the activity of the pulmonary stretch receptors of the Hering-Breuer inflation reflex may also be altered by minute doses of some drugs.

Experiments of Heubner & Viessmann (136) showed that *p*-chloroethanolamine provokes a reflex apnea which is not of carotid sinus origin, but is abolished by vagotomy; this drug however does not cause a fall in arterial pressure and heart rate. Kramer (137) also studied the respiratory effects of this compound and tried to elucidate the mechanism of this reflex apnea. According to this author the apneic effect, which is not accompanied by other depressor phenomena, is attributable to an action upon chemosensitive receptors lying closely to the pulmonary artery; whether or not these receptors are identical to those influenced by the veratrum alkaloids is still unsettled. Although it has not been definitely proved, Bucher (138) believes that these chemosensitive receptors are unspecific and identical with stretch receptors; stimulation of these stretch receptors also induces apnea.

According to Kottegoda & Mott (66) intravenous injection of appropriate doses of 5-hydroxytryptamine (HT) in cats, also elicits an apneic response from reflex origin; this apnea closely resembles that caused by the amidines, but is complicated by additional factors. The authors conclude that stimulation of pulmonary stretch receptors is unlikely to play a dominant rôle in the production of reflex apnea by HT. On the other hand Schneider & Yonkman (65) observed a stimulation of the pulmonary stretch receptors with HT as intense as that caused by veratridine. There are, however, still some points which require confirmation, before concluding that HT provokes apnea by the same type of reflex mechanism as that which is excited by the amidines [Dawes & Comroe (4)].

### Abdominal Receptors

*Stomach and intestines.*—The sensory nerves in stomach and intestines have hitherto been passed over, mainly because of the assumption that no such structures can be detected in these viscera. In recent years however it

has been established, chiefly by Japanese physiologists, that sensory receptors and nerve fibres are present in some abdominal organs (Seto, 139; Sato, 140).

According to Ohi (141, 142), not only autonomic sympathetic and parasympathetic nerve fibres, but also sensory nerve endings may be seen in the stomach and duodenum walls of white rats. Transmission of sensory stimuli is effected by way of cerebrospinal nerve fibres. The sensory fibres may be histologically recognized and are easily distinguishable from vegetative structures. The reason for the existence of these sensory visceral receptors presumably consists, physiologically, in the formation of a reflex arc which promotes secretion of digestive fluids and, pathologically, in the perception of peculiar pains.

Iggo (143) recorded the impulses in afferent fibres of the cervical vagus of anaesthetized goats during distension of certain parts of the stomach. Gastric tension receptors have been located near the junction of the esophageal groove with the reticuloruminal sac. From the behaviour of the gastric receptors during distension, pulling, or contraction of the walls, it is suggested that these receptors are not acting as so-called volume receptors, but are tension receptors.

A method of locating the receptors of visceral afferent structures has been recently described by Paintal (127). Phenyl diguanide, which is capable of stimulating such receptors, was injected into different vascular channels of the chloralosed cat. The observed effects on the activity of afferent nerve fibres isolated from the right vagus were recorded by means of a cathode-ray oscillograph. It was found that most receptors are located in the wall of the stomach, some in the intestines. The stimulating effects of the drug were completely abolished by bilateral vagotomy below the diaphragm.

Further experiments of Paintal (144) on gastric receptors in the cat have shown the existence of 14 stretch receptors in the stomach; most of them are localized in the pyloric area; their afferent pathways run in the vagus. The normal function of the gastric stretch fibres is to signal the degree of distension of the stomach. It is very likely that these receptors—probably situated in the smooth muscles—are responsible for the immediate feeling of satiation of hunger and thirst.

The location and specific properties of other visceral vagal receptors were also investigated by Paintal (128). It was demonstrated that various abdominal and thoracic receptors sensitive to phenyl diguanide may be encountered; two of these are located in the intestines and two are probably of pulmonary origin. Although their normal physiological functions are not known, it would appear that these visceral vagal receptors are primarily sensitive to drugs and that their stimulation is not secondary to contraction or relaxation of the smooth muscles.

According to Dreyer's investigations (145) on rebound effects in the cat's intestine, epinephrine and some other sympathomimetic amines produce an inhibition of intestinal activity which is followed by an increased motility

or "rebound." In intact cats as well as on excised intestine it was shown that the Auerbach's plexus is responsible for the rebound, since this rebound effect is abolished by large doses of hexamethonium or by degeneration of the ganglion during storage of the intestine at 4°C.

*Bladder.*—Action potentials from afferent fibres of the pelvic plexus of anaesthetized cats, during distension of the urinary bladder, have been recorded by Iggo (143). As the intravesical pressure rose there was a burst of impulses, the frequency of which reached a maximum of 20 to 30/sec. and then fell sharply although the pressure was still rising. During rhythmic contractions of the bladder, impulses were recorded in the afferent units during the rise in intravesical pressure. The receptors were located at the neck of the bladder and could be stimulated by pulling on strips of the bladder wall. Evidence has been found which suggests that these receptors are tension receptors in series with the muscle.

*Oviposition.*—Experimental investigations to consider how far reflex bearing-down is essential for the accomplishment of oviposition in fowl have recently been carried out by Sykes (146, 147). It was observed that distension of the vagina results in hyperpnea, contraction of the abdominal muscles, and rise in abdominal pressure. This reflex is abolished by general or local anaesthesia (of the vagina), and in some cases by spinal cord section. The receptor structures of the reflex are situated along the length of the vagina. It appears that in the fowl and in some mammals the abdominal muscles are essential for normal delivery, and these muscles are controlled by a reflex arising from distension of the vagina.

*Muscular receptors.*—Experimental investigations on muscle metabolism and the regulation of breathing, carried out by Ramsay (148) on anaesthetized dogs, showed that an increase in the metabolism of the limb provokes an increase in pulmonary ventilation; this effect is undoubtedly mediated by nerves, since no change in ventilation occurs after section of the sciatic and femoral nerves. It is suggested that there are muscular receptors sensitive to changes in metabolic activity; these may be the site of origin of the "exercise stimulus."

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# PITUITARY-ADRENAL RELATIONSHIPS[1,2]

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This review is devoted to a consideration of the mechanism or mechanisms by means of which the secretion of ACTH and, consequently, that of the adrenal steroids, is adjusted to the needs of the organism. In point of fact these studies on the regulation of ACTH secretion are part of the larger and important problem of the manner of regulation of the secretion of all anterior lobe hormones. Consequently, it is impossible to avoid mention of at least a certain number of studies which, while not directed specifically to the anterior pituitary-adrenal cortical relationship, nevertheless indicate that there exists a pattern of regulation that is common to all the anterior lobe hormones.

The problem, around which so much work has been done in recent years, may be outlined as follows. It is agreed that the anterior pituitary is devoid of secretory nerve fibres and that therefore the mechanisms of secretion must be attributed to humoral factors that reach the gland through its blood supply. In the case of the anterior pituitary this has several unique characteristics inasmuch as branches of the internal carotid not only penetrate directly into the substance of the organ but also enter the median eminence. The latter branches break up into a capillary network which reunities to form portal vessels that then run down the pituitary stalk for varying distances before breaking up again into a second capillary network that supplies the secretory cells (1, 2).

This dual type of blood supply offers two different opportunities for a humoral agent to reach the anterior lobe. The first places the secretory cells in contact with any agent that has entered the systemic blood at distant parts of the body, while the second, the portal system, enables this organ to establish a vascular link with any nerve fibres whose axons terminate in the median eminence. Since it is now well known that certain axons do liberate highly active humoral agents at their terminations, the possibility exists that hypothalamic centers sending their axons into the median eminence may influence anterior lobe secretion by means of a humoral agent that is carried into the gland by the portal vessels. Neurohumoral control of the secretory function of cells or organs is, of course, a well-established fact, even though in the case of the hypothalamic-hypophyseal link the distance over which the supposed humoral agent is carried by vascular channels is exceptionally far. Indeed, in all other instances of neurohumoral transmission the contact

[1] The survey of the literature pertaining to this review was concluded in June, 1955.

[2] The following abbreviations will be used: ACTH, (adrenocorticotrophin); ADH, (antidiuretic hormone).

between agent and receptor does not require the entry of the former into the blood itself.

The hypothesis that there is a vascular connection between elements in the hypothalamus and the secretory cells of the anterior lobe is not a new one, although is recent years it has been given new prominence largely through the brilliant experimental work of Harris and his colleagues. This work has been extensively reviewed by Fortier & Harris (3) and others (4 to 8). In brief, Harris and his colleagues regard the vascular contact by means of the portal vessels as essential not only for ACTH release but also for that of the gonadotrophic, thyrotrophic, and possibly other anterior lobe hormones. Since these vessels serve merely as a vascular link between hypothalamus and endocrine organ, it follows that the really essential elements in this system are the hypothalamic centers themselves whose terminal axons furnish the transmitting agent. The location and number of these centers is still a matter of discussion, as is the question concerning the absolute necessity for an intact hypothalamic-hypophyseal link for an increased rate of anterior lobe secretion. Indeed there are some investigators who, while admitting that the evidence for some kind of central nervous control of anterior pituitary activity is justified by the experimental facts, nevertheless believe that this is not the only mechanism and, indeed, regard the hypothalamic component more as a fine adjustor of secretory activity than the essential one. There is, in fact, a considerable body of evidence to indicate that the secretions of the endocrine organs controlled by the anterior pituitary exert a major effect on the rate of secretion of the trophic hormones themselves, to the extent that the rate of release of the latter is inversely proportional to the blood level of the former. However, it should be pointed out that the site of action of a changing blood level of, for instance, the adrenal steroids has not been determined, although it has been assumed to be on the anterior lobe itself. It could also be on the appropriate hypothalamic center. If this were the case then the view that these centers are the essential component in the regulatory system would be considerably strengthened.

Another question which follows from the fact that the anterior pituitary secretes several hormones is whether all these hormones are under the control of a single transmitter or whether such an hypothesis does not carry with it the deduction that there are as many discrete hypothalamic centers and neurohumoral transmitters as there are anterior lobe hormones themselves.

This review will attempt to consider the more recent experiments that bear on some of the questions raised in these prefatory remarks. No attempt has been made to cite every paper that might have some bearing on this topic. For such omissions this reviewer must take the responsibility.

## The Effect of Pituitary Stalk Section and Hypothalamic Lesions on the Secretion of ACTH

*Stalk section.*—One of the key experiments cited by Harris in support of his view on the importance of the hypothalamic-hypophyseal vascular link,

is that section of the pituitary stalk will have only a temporary and often brief effect on anterior lobe secretion unless steps are taken to prevent the regeneration of the hypophyseal portal vessels. This, it is stated, is achieved if a paper plate is placed between the cut ends of the stalk. DeGroot (9) now confirms this observation using mice. He reports that the lymphopenia following emotional stress or the injection of epinephrine is only temporarily abolished by stalk section alone, but can be prevented permanently by the interposition of a paper plate between the severed ends of the stalk.

The validity of the argument that the prevention of regeneration of the portal vessels does not interfere to any marked degree with the blood supply to the anterior lobe but merely prevents the passage of the neurohumoral agent has not gone unchallenged. Harris himself has noted that varying degrees of infarction may follow stalk section, while Barrnett & Greep (10) are of the opinion that in the rat devascularization of the anterior lobe is the most important element in the failure of adrenal and thyroid glands of such animals to respond to cold stress. Tang & Patton (11), who used guinea pigs, state that while in some animals stalk section may be followed by glandular atrophy, in others but little change is observed in adrenal, testes, or thyroid weight. The absence of significant adrenal atrophy after stalk section has also been reported by others. Tang & Patton also note that in some of their animals normal falls in adrenal cholesterol were observed after exposure to cold, even though they could not detect any regeneration of the portal vessels.

Keller *et al.* (12) destroyed in dogs the proximal portion of the hypophyseal stalk and the adjacent area of the median eminence and hypothalamus. Subsequent exposure of the animals to heat, cold, or hypoglycemia still produced eosinopenia, presumably attributable to adrenal cortical discharge, even though they state that the lesions destroyed all vascular channels and neurons linking the hypothalamus and hypophysis. Such animals did not show any signs of adrenal atrophy nor of adrenal insufficiency since diabetes developed to the usual degree after total pancreatectomy.

An equally sharp difference of opinion as to the effects of stalk section on reproductive activity is evident. Thomson & Zuckerman (13, 14) find that light-induced estrus in the ferret is not affected by stalk section even when regeneration of the portal vessels is prevented by means of the placing of a paper plate between the cut ends. Donovan & Harris (15) repeated these experiments and state that in their experience estrus only occurs when there is regeneration of the portal hypophyseal vessels. However, Thomson & Zuckerman (16) have demonstrated a wax reconstruction of one of their animals in which the anterior lobe was completely separated from the median eminence by a massive barrier composed of cellulose, new bone, and connective tissue. In spite of the fact that the remains of the portal system now emptied into vessels above the barrier, estrus had occurred on exposure of the animal to light.

It is evident that these deep-seated differences of opinion rest on two points: (*a*) can it always be demonstrated that there has been no re-establish-

ment of a vascular connection between the median eminence and hypophysis and (*b*) if such severance is complete, to what extent is the failure of anterior lobe function to be attributed to ischemia of the gland rather than to the absence of a neurohumoral agent. Neither of these questions has been completely answered.

It may be remarked in the case of the adrenal cortex that section of the stalk, while it may interfere to some degree with the capacity of the anterior pituitary to secrete ACTH, nonetheless is compatible with nearly normal adrenal cortical function as judged by the absence of any reports of adrenal insufficiency in such animals or by any striking decline in adrenal size. All investigators are agreed that neither stalk section nor hypothalamic lesions produce the degree of adrenal atrophy observed after complete hypophysectomy.

*Hypothalamic lesions.*—Since stalk section is bound to interfere, at least for a time, with the blood supply to the pituitary, it would seem that the demonstration of the participation of hypothalamic centers in the regulation of ACTH secretion could best be made by the selective destruction of these centers in such a way that extensive injury to the portal hypophyseal vessels was avoided. Earlier, Harris and his colleagues had found that lesions in the posterior hypothalamus prevented lymphopenia in restrained rabbits. Hume and his colleagues on the other hand found in the dog that the eosinopenia in response to trauma was abolished by anterior hypothalamic lesions.

The development of technics for the estimation of the 17-hydroxysteroids in the blood of the adrenal vein has given an improved method for the recognition of activation of the pituitary-adrenal system and has been applied to the problem of the regulation of ACTH secretion by several laboratories. Laquer *et al.* (17) and McCann *et al.* (18) found in cats that lesions which destroyed the median eminence and adjacent hypothalamus were associated not only with a decreased eosinopenia after laparotomy and epinephrine injection but also with a decline in the quantity of steroids in the adrenal vein blood. Using rats, McCann (19, 20) found that destruction of the median eminence blocks the usual fall in adrenal ascorbic acid after unilateral adrenalectomy in spite of the fact that no adrenal atrophy was observed. However, stalk section not only blocked this response but was associated with adrenal and testicular atrophy.

Anand *et al.* (21) placed lesions in various parts of the hypothalamus of rats and observed their effect on the eosinopenic response to painful stimuli (10 per cent NaCl subcutaneously), laparotomy, and the injection of epinephrine. They conclude, as have others (Long, and Fortier), that there exists a dual type of control of ACTH secretion, only one of which is dependent on the hypothalamus. They base this conclusion on their observation that while certain hypothalamic lesions abolished the fall in esosinophiles usually seen 1 hr. after laparotomy or a painful stimulus, such lesions did not alter the eosinopenic response to epinephrine. The hypothalamic lesions that blocked the response to the first two stresses were located bilaterally in the medial

part of the hypothalamus in the plane of the paraventricular nuclei and the area just posterior to it. This is approximately in the area of the anterior and medial part of the median eminence in the plane of the ventro-medial nuclei. Lesions in the paraventricular nuclei were without effect as were those in several other areas of the hypothalamus.

A comparison of these experiments with those of McCann is of interest. Both investigators used rats and placed the lesions by means of a stereotactic instrument. Both are agreed that responses to painful stimuli (laparotomy, unilateral adrenalectomy, or injection of 10 per cent NaCl) are abolished by lesions that destroy a portion of the median eminence. Anand *et al.*, however, find that the response to epinephrine is unchanged, while Laquer, McCann *et al.* find in cats that this too is decreased by somewhat comparable lesions. Harris & Fortier in a recent review (3) cite previously unpublished experiments in rabbits which indicate that while the lymphopenia induced by cold and restraint were greatly reduced or abolished by stalk section, that brought about by laparotomy or the injection of epinephrine was only slightly less than in intact animals. Using the decline in adrenal ascorbic acid as a criterion of ACTH release, it was found that stalk section had little if any effect on the decline in this adrenal constituent that occurs after unilateral adrenalectomy.

Ganong, Gold & Hume (22) found that lesions in the median eminence of dogs abolished the usual increase in 17-hydroxycorticoids that follows immobilization of the animals, while Hume & Nelson (23) report that trauma produces no increase in the blood ACTH (determined by the effect of infusion of blood of the traumatized animal on the 17-hydroxycorticoid output of adrenals of an hypophysectomized dog) in dogs with similar lesions in the median eminence.

Ganong & Hume (24) found that the hypertrophy of the right adrenal of the dog that occurs some two weeks after removal of the other gland is prevented by destruction of a half or more of the median eminence. Lesions that did not involve this area were without effect. In a converse type of experiment Ganong & Hume (25) performed left adrenalectomy and then treated the dogs with large amounts of cortisone (50 mg. a day) for two weeks. Atrophy of the remaining adrenal occurred whether or not the median eminence had been destroyed, leading them to conclude that, while this area was necessary for the secretion of ACTH after stress, it was not involved in the decrease in the secretion of this hormone that is induced by large quantities of cortisone. This would suggest that the suppression of ACTH and adrenal atrophy under these conditions is attributable to a direct effect of the steroid on the anterior lobe itself, a point of no little importance since, in effect, it admits the possibility of a dual regulation for the secretion of this trophic hormone. The experiments reported by Kallman & Gordon (26) are of interest in this regard. These investigators placed small pellets of cortisone in direct contact with the pituitary gland of rats. Sixty days later the animals were sacrificed, at which time it was observed that while there

was no atrophy of the adrenals, thyroid, or gonads, nevertheless the areas of the pituitary in close contact with the steroid showed increases and other changes in the basophiles. They conclude that the action of the hormone was directly on the anterior pituitary cells, but since it did not involve a sufficient quantity of the gland the reduction in the secretion of the trophic hormones was not sufficient to affect the various target organs. These experiments indicate, as do those of Ganong & Hume, that the adrenal steroids inhibit the secretion of ACTH by their effect on the gland itself and do not do so by their suppressive effect on hypothalamic nuclei.

While earlier work had indicated that hypothalamic lesions that did not involve the median eminence might interfere with ACTH secretion, it would appear from more recent studies in three species of animals that the most effective lesions are those that destroy this structure to a greater or lesser degree. Since it is in this area that the point of contact between the nervous and vascular elements is believed to occur, the question will still arise as to whether such lesions are effective (*a*) because they reduce the blood supply of the gland, or (*b*) because they prevent the passage of a humoral agent to the gland from the hypothalamus. However, while stalk section is not infrequently associated with adrenal atrophy, lesions in the median eminence only produce slight, if any such, effect. Nevertheless, the nature of the anatomy of this area is such that it will always be difficult to decide how much impairment of the blood supply to the anterior lobe has occurred, as well as to assess the effect of such impairment on the capacity of the gland to secrete ACTH.

### The Effect of Stimulation of the Hypothalamus and Other Areas of the Central Nervous System on ACTH Secretion

DeGroot & Harris (27) have reported that electrical stimulation of the mammillary body or posterior tuber cinereum produced lymphopenia in conscious rabbits. Porter (28), using monkeys, has also shown that electrical stimulation of the median eminence, medial tuberal, mammillary, lateral posterior, and lateral tuberal areas brought about significant falls in the number of circulating eosinophiles. He also reports that excitation of the orbital surface of the frontal lobe was followed by eosinopenia of an equal degree, while slightly smaller effects were obtained by stimulation of the prefrontal cortex. Section of the spinal cord at the level of the seventh cervical vertebra 48 hr. beforehand did not alter these responses, which were, however, abolished by section of the pituitary stalk. Of equal interest is the observation that stimulation of other areas of the cortex diminished the eosinopenia that follows the injection of epinephrine or operative trauma. Such as area was found in the hippocampal region. He concludes that the hypothalamic centers may be excited or inhibited by impulses arising in the central nervous system and that the final common pathway to the anterior lobe is probably through the hypophyseal portal system.

Anand & Dua (29) have carried out somewhat similar types of experiment using cats in which electrodes were permanently implanted in various regions of the hypothalamus. They found that stimulation of the medial part of the anterior and middle hypothalamic regions, particularly the anteromedial region of the median eminence, was followed by a fall in circulating eosinophiles.

The studies of these investigators together with the earlier work of Harris and his colleagues indicate that stimulation of the medial and posterior regions of the hypothalamus, as well as of the median eminence, increases the secretion of ACTH, at least as judged by the decline in the level of blood lymphocytes and eosinophiles. Furthermore, the evidence would indicate that the effects of hypothalamic stimulation are not entirely a consequence of excitation of long descending pathways that bring about secretion of the adrenal medullary hormones, although the reinforcement of hypothalamic or hypophyseal activity by these agents cannot be excluded as a factor in the intact animal.

### Humoral Agents Suggested as Regulators of ACTH Secretion

Since it is generally agreed that the secretory activity of the anterior lobe can only be affected by humoral means, there has been much work done in an attempt to identify the particular agent or agents that influence the gland in this manner.

Two views as to the manner of access of this agent (or agents) to the anterior lobe cells have been suggested. The first, arising from the work of Harris and his colleagues, is that nerve fibres terminating in the median eminence come into contact with the hypophyseal portal blood vessels and release at their endings a specific agent that passes directly to the receptive cells of the gland. Since there is at the present time substantial evidence only for the release of sympathin or acetylcholine at nerve endings, it is natural that both of these should have been implicated as playing a similar role as transmitters to the pituitary. On the other hand, there are those who believe that neither of these substances is the responsible factor in the final pathway although they may participate in the transmission of impulses to the hypothalamus from other parts of the central nervous system. Consequently, it has been suggested that either a presently unknown "hypothalamic hormone" is involved or else that a principle associated with the posterior lobe system is the effective agent.

The second view put forward is that the humoral agents responsible for activation of the anterior lobe need not necessarily arise in the hypothalamus and be transmitted by the portal system, but may have their origin in more distant parts of the body where by entering the systemic blood they eventually reach and affect the anterior lobe without the intervention of elements of the central nervous sytem. The substances that have been suggested as exerting such an effect are the hormones of the adrenal cortex and of the

adrenal medulla. The evidence both for and against the participation of the various substances that might function as humoral links in the activation of ACTH secretion may now be considered.

*Epinephrine and norepinephrine.*—The fact that so many circumstances associated with an increased rate of secretion of ACTH are also those known to cause an activation of both peripheral and central adrenergic elements of the autonomic nervous system has led to a rather thorough investigation of the possibility that epinephrine and norepinephrine may be the humoral link responsible for the release of ACTH. In considering this question it must be rememberted that these agents are released not only by the adrenal medulla directly into the systemic circulation but also are liberated at the terminations of certain of the neurons themselves, where presumably a relatively high but evanescent concentration may be present during the excitatory period.

In keeping with so many other questions concerning the regulation of ACTH secretion there is at present a marked difference of opinion as to the importance of epinephrine and norepinephrine in the regulation of ACTH secretion. It has been reported many times that the subcutaneous or intravenous injection of epinephrine, and to a lesser degree, of norepinephrine, causes in the intact animal the following effects; a depletion of adrenal ascorbic acid and cholesterol; shrinkage of lymphoid tissue; a fall in the number of circulating lymphocytes and eosinophiles; and a depletion of the adrenal cortical lipides. These effects are all indicative of stimultation of the pituitary-adrenal system and are not observed in adrenalectomized or hypophysectomized animals.[3] Furthermore, Vogt (39) has shown that the intravenous injection of epinephrine in the dog is followed by a prompt and sustained increase in the quantitiy of adrenal hormones in the effluent blood of

[3] Some exception can be taken to the statement that epinephrine is without effect on the eosinophiles of adrenalectomized animals since there are several reports that in Addison's disease or in adrenalectomized humans, eosinopenia is still evoked by this hormone (30, 31, 32). In some instances the individuals were receiving, or had recently been given, maintenance doses of cortisone. Henry, Oliner & Ramey (33) observed that epinephrine causes eosinopenia in adrenalectomized dogs only when given along with cortisone or if very large doses of it were given. Such effects are stated to be an example of the "permissive" action of the adrenal steroids. However, in man at least, the presence of residual quantities of cortical tissue cannot be excluded.

Moreover, Jakobson & Hortling (34) observed no fall in the eosinophiles after the injection of epinephrine into recently adrenalectomized or hypophysectomized rats, a finding that has been repeatedly observed in this species in the author's laboratory.

Although Vogt (35, 36) has reported that the acute or chronic injection of epinephrine will cause depletion of adrenal cortical lipids in the rat and Zeckwer (37) has found that the daily injection of this hormone for three to seven days not only brings about adrenal enlargement but also involution of the thymus, Coutinho, Baker, & Ingle (38) state that they failed to observe any histological evidence of pituitary-adrenal stimulation under these conditions.

the gland, at least as judged by the capacity of the plasma obtained from this blood to protect adrenalectomized rats against cold. Okinaka and his colleagues (40, 41) also found an increase in the cortical hormone content of the adrenal vein blood of dogs (estimated by the Heard-Sobel method) after stimulation of the peripheral end of the splanchnic nerves or after the injection of epinephrine. However, it is their contention that such an increase occurs only in the adrenal vein blood of the gland on the side on which the splanchnic nerve is stimulated, and not in the blood from the gland on the unstimulated side. They also report that a similar increase in blood adrenal steroids follows splanchnic stimulation of the nerve or epinephrine injection into hypophysectomized dogs and, in consequence, suggest that the secretion of adrenal steroids may be under the control of a peripheral mechanism as well as that of ACTH. Since studies on the isolated perfused ox adrenal have shown epinephrine to be without effect on the output of adrenal steroids, these results require extension and confirmation. Nevertheless, it has been shown that while hypophysectomy greatly reduces the quantity of 17-hydroxy steroids it only has a comparatively small effect on the output of aldosterone (42, 43). However, it is unlikely that the increases observed by Okinaka *et al.* in hypophysectomized animals could be attributed solely to aldosterone.

Using a cross circulation technique in dogs, Malmejac, Gross & Nereme (44), on the basis of changes in the lymphocyte count, concluded that the splanchnic nerve has only an indirect effect on adrenal cortical secretion and that this is effected through the liberation of epinephrine.

Farrell & McCann (45), Gemzell (46), and Bozovic & Milkovic (47) all report that epinephrine increases the quantity of ACTH in the blood of normal rats, but, while Gemzell states that this is accompanied by a fall in the content of ACTH in the pituitary, Bozovic & Milkovic observed an increase. In adrenalectomized rats Gemzell (46, 48) finds that epinephrine, but not norepinephrine, reduces the blood level of ACTH. Long & Brodish (49), using a cross circulation technique in which the blood of an intact rat is exchanged with that of an hypophysectomized partner, also found that the injection of epinephrine increased the blood level of ACTH, as judged by the effect of the blood from the intact rat on the adrenal ascorbic acid of the hypophysectomized animal.

Although the earlier experiments of Vogt and Long indicated that epinephrine could stimulate the secretion of ACTH it was never suggested that the prior release of this hormone from the adrenal medulla was essential for the activation of the anterior lobe. Apart from the fact that demedullated adrenals undergo regeneration, Vogt (50) has shown in rats with demedullated glands that, although no rise in blood epinephrine follows their subjection to sensory nerve stimulation or bleeding, nevertheless the adrenal ascorbic acid falls. Hodges (51) has also observed that laparotomy brings about a fall in adrenal ascorbic acid in rats with demedullated adrenals.

While it is an undoubted fact that loss of the adrenal medullary secretion

does not interfere with the release of ACTH under all circumstances, nevertheless there are some in which the response is diminished if the secretion of epinephrine is prevented. Vogt (35), for example, has reported that while adrenal denervation did not prevent the loss of adrenal lipids following bleeding or exposure to heat or cold, it usually prevented the loss that occurs after the administration of insulin.

The question as to the possibility that epinephrine and norepinephrine can serve as neurohumoral transmitters between the hypothalamus and pituitary is not settled in the negative by the demonstration that presence of the adrenal medulla is not essential for ACTH release. For it is a well-known fact that the local release of these substances at the termination of axons still occurs in its absence, and, while the simultaneous release of these substances from the adrenal medulla may extend and prolong their local effects, it is no more necessary to regard the medulla as essential for an effect on the anterior lobe than it is for a response from any other organ with an adrenergic innervation.

In an attempt to prevent the release, or the effects after release, of epinephrine and norepinephrine, whatever their origin in the body, several investigators have recently used adrenergic blocking or adrenolytic agents. In the main these results are indecisive, since in most instances the injection of the blocking agent is in itself a rapidly acting stimulus to ACTH release. Thus, Hamilton (52) finds that phenoxybenzamine (Dibenzyline) has such an effect and furthermore apparently does not block the fall in adrenal ascorbic acid after epinephrine injection. Ohler & Sevy (53, 54) report that while this drug inhibits the response to epinephrine it does not prevent the fall in adrenal ascorbic acid after unilateral adrenalectomy. Consequently they conclude that endogenous epinephrine does not play a primary role in ACTH release.

On the other hand Jakobson & Hortling (34) state that dihydroergotamine not only prevents the effects of subcutaneously-administered epinephrine in depressing the eosinophile count in rats but also blocks the fall that follows exposure to cold. Sawyer (55) found in the rat that N(9-fluorenyl)-N ethyl-$\beta$-chlorethylamine (SKF 501) and 2-dibenzylaminoethanol (D-ol), both analogues of N,N-dibenzyl-$\beta$-chloroethylamine (Dibenamine) block the fall in eosinophiles that occurs 1 hr. after the injection of epinephrine or formalin, while not affecting the fall that occurs 4 hr. after such injections. This delay in the response, or rather the abolition of the immediate response, is reminiscent of the effects on the stress responses observed by Anand *et al.* (21) (cf. above) after certain hypothalamic lesions in rats, and also of those reported by McDermott *et al.* (56) after both hypothalamic injury and high spinal cord section. These results suggest, as do many other experiments, that more than one mechanism exists for the release of ACTH.

Aron, Chambon & Voisin (57), using the drug chlorpromazine (4560 R.P.; Largactil), injected 2 to 5 hr. before exposure of rats to unilateral

adrenalectomy or formalin injection, found that it blocked the usual fall in adrenal ascorbic acid but did not interfere with the response to ACTH.

Perhaps the most puzzling feature of the effect of epinephrine on the pituitary-adrenal system is its complete lack of effect on the blood levels of adrenal 17-hydroxy steroids. This in spite of the fact that so many indirect methods indicate that it has brought about a release of ACTH. Thus, Kelley *et al.* (58), Nelson (59), Martin & Pattee (60), Spengler (61), Wolfson (62), Meyer (63), Sandberg *et al.* (64), Ely *et al.* (65), and Hunter & Bayliss (66) all report that, while in man epinephrine produces marked eosinopenia, it has only an insignificant effect on the blood level of these adrenal steroids. Even in the rat, where the fall in adrenal ascorbic acid is so evident after epinephrine, Bacchus (67) could not detect any increase in blood adrenal steroids. In most of these reports the injection of ACTH brought about distinct and often marked increases in the blood steroids. Unless we are willing to admit that the fall in adrenal ascorbic acid and of the circulating lymphocytes or eosinophiles are useless as indicators of ACTH release, some other explanation of the failure of the blood adrenal steroids to increase after the injection of epinephrine must be found. One explanation might be that epinephrine has a twofold effect on the pituitary-adrenal system. In the first place it increases the rate of adrenal cortical secretion by stimulating the release of ACTH. However, it also produces an increase in the over-all metabolic rate in consequence of which the organism "utilizes" the adrenal steroids at an increased rate. Thus, increased production is counterbalanced by increased utilization, and little or no change in the blood level occurs even though the increased needs of the organism for these hormones have been met by an increased rate of secretion. Such a situation would of course be quite different from that produced by giving an excess of ACTH to an animal in the resting state. It would also be in agreement with the observation that norepinephrine, which has only a feeble calorigenic effect, is also much less active than epinephrine in causing a loss of adrenal ascorbic acid. On the other hand, if epinephrine produces its effects by increasing the peripheral utilization of adrenal steroids, then it can only be assumed, as Sayers has suggested, that the lowered blood level of adrenal steroids is an effective stimulus to ACTH secretion.

Other explanations of the mechanism of action of epinephrine have been advanced. McDermott *et al.* and Fortier found that the direct injection of small quantities of epinephrine into the eye of an hypophysectomized rat which contained a viable graft of anterior lobe was followed by a fall in eosinophiles. This indicates an effect of this hormone on the anterior lobe itself, a possibility that is supported to some degree by the work of Keller & Roberts (68), who have found that the rate of respiration and aerobic glycolysis of isolated anterior pituitary from rats is transiently enhanced by the administration of epinephrine.

Porter (69), however, has published evidence that epinephrine injection is followed by increased electrical activity in the posterior hypothalamus

which was attributed to the stimulation of these centers by this hormone. Since the areas in which this activity occurs coincide with those whose destruction is followed by an absence of eosinopenia after epinephrine injection, Porter has suggested that epinephrine produces its effects on ACTH secretion by stimulating the hypothalamic centers rather than the gland itself. An unexplained finding in these experiments is that the injection of insulin not only also increased electrical activity in these areas but did it as rapidly as epinephrine itself. In guinea pigs Green & Morin (70) state that the slow rhythms in the anterior hypothalamus and the fast rhythms in the posterior hypothalamus are not influenced by epinephrine, nor by such stimuli as light, sound, or strong odors. However, Feldberg & Sherwood (71) state that in cats the intraventricular injection of epinephrine or norepinephrine produces a condition resembling light pentobarbital anesthesia, an effect also reported by Leimdorfer (72) some years ago.

Rose (73) has published a paper in which he reports rather unusual effects following the intrathecal injection of epinephrine, in amounts of 1 to 5 mg., in man. While no change occurred in blood pressure or heart rate, the blood glucose was elevated for many hours. In addition, there was a marked decrease in eosinophiles and an increase in the uric acid/creatinine ratio. Even more remarkable is his report that such injections into individuals with rheumatoid arthritis or asthma was followed by marked but temporary symptomatic relief. He suggests that objections to the importance of epinephrine in the pituitary-adrenal response lose much of their validity when the possibility of local release in the hypothalamus or pituitary is considered.

Earlier, it was stated that the fact that ACTH can still be released in adrenal-demedullated animals did not in itself justify the conclusion that epinephrine could have no role in humoral transmission from the hypothalamus to the anterior pituitary. The difficulty in attempting to reproduce the local concentration of epinephrine or norepinephrine by systemic injection is emphasized by Von Euler (74), who calculates that in the spleen the concentration of these substances at the nerve endings may be between 3 and 30 mg. per gm., an amount that is about the same as the concentration of catechol amines in the adrenal medulla.

Vogt (75) has recently reported the results of her studies on the distribution of sympathin in the central nervous system. She finds that the hypothalamus, both anterior and posterior, along with the area postrema, contains the largest quantities of these amines. Furthermore, the concentration in the hypothalamus is reduced by subjecting the animals to insulin shock, a procedure that also reduces their content in the adrenal medulla.

Apart from the possibility of the local release of epinephrine or norepinephrine at the termination of the axons of hypothalamic nuclei, there is ample evidence that stimulation of certain areas in the hypothalamus brings about release of the adrenal medullary hormones presumably by activation of long descending pathways to the spinal cord and splanchnic nerves. Recent papers on this subject are those of Brauner *et al.* (76), Brucke, Kaindl

& Mayer (77), Redgate & Gellhorn (78), Suzuki & Arai (79), and Conley & Gellhorn (80).

An adrenergic mechanism has also been proposed as the final step in the release of the gonadotrophic hormones. Markee *et al.* (81) have reviewed their extensive studies on the relation of the nervous system to the regulation of the sex cycle. They report that the direct application of epinephrine to the pituitary, or its introduction into the cerebral ventricles, is followed by ovulation in the rabbit. Sawyer (82) also finds that the intravenous injection of rather large amounts of epinephrine into rabbits, protected against its lethal effects by previous atropinization, is followed by release of leuteinizing hormone. Since the amounts needed to produce ovulation when given intraventricularly are very much less than those required when given by vein, the presumption would be that the increase of the local concentration of the hormone in some area of the nervous system is the important factor.

Donovan & Harris (83) do not agree that epinephrine is an important agent in the mechanism of ovulation in the rabbit. They injected it directly into the area of hypophyseal portal vessels and state that, while the bitartrate of epinephrine and norepinephrine cause ovulation in a significant number of experiments, adjustment of the solutions to neutrality abolished this response. They conclude that the positive effects were due to the acidity of the solution although they recognize that in neutral solutions at the slow rate of infusion employed (0.0002 ml. per min.) an increased rate of destruction of the amines could be a factor. They attempted to answer this by increasing the amounts infused from 120 μg. to 150 μg. However, the question may well be asked why the entry of an acid solution into the portal vessels in itself would cause ovulation. They do not report whether the infusion of solutions of the same acidity of those of the epinephrine bitartrate solutions used had any effect.

*Histamine.*—Harris, Jacobsohn & Kahlson (84) have shown that the median eminence contains unusually high quantities of histamine, a substance that is quite active in causing depletion of adrenal ascorbic acid and circulating eosinophiles. Consequently, some attention has been given to the possibility that this substance could serve as a humoral agent in the release of ACTH. Since histamine injections also stimulate the adrenal medulla, an attempt has been made to differentiate between its effect on epinephrine secretion and those effects that might be specific to it. Guillemin & Fortier (85) found that the effects of cold, sound and immobilization were not blocked by the anti-histaminic agent Phenergan [promethazine; 10-(2-dimethylamino-1-propyl) phenothiazine]. Semonsen & Sawyer (86) found that the fall in eosinophiles that occurs 1 hr. after the subcutaneous injection of histamine acid phosphate into anesthetized rats is prevented by prior injection of the adrenergic blocking agent SKF 501. The Dibenamine analogue D-ol, which does not prevent the effect of epinephrine on the eosinophiles, also blocked the immediate (1 hr.) response to histamine, indicating that it had prevented the reflex secretion of epinephrine. Neither substance

had any effect on the delayed (4 hr.) fall in eosinophiles. In anesthetized rats with demedullated adrenals, histamine was ineffective in bringing about the usual fall 1 hr. after subcutaneous injection but did not prevent the delayed fall. However, the effect of the intravenous injection of histamine was not altered either by the blocking agents or by adrenal demedullation. They conclude that part of the effect of histamine is attributable to the reflex secretion of epinephrine, but that, in addition, there appears to be a direct effect of this agent either on the pituitary or in the central nervous system.

Guillemin (87) in a recent paper has pointed out some of the difficulties in attempting to relate the effects of a blocking agent on exogenously administered agents such as epinephrine to their role as humoral transmitters to the pituitary. He first adapted rats to the nonspecific effects of the pharmacological antagonists by daily injection of the agent for several days. He observed in such adapted animals that the effects of exogenous epinephrine on the adrenal ascorbic acid were blocked by SKF 501, the effects of norepinephrine were blocked by Dibenzyline, and the effects of acetylcholine were blocked by atropine. However, subsequent exposure of these adapted animals to a systemic stress such as formalin injection or an emotional stimulus such as bodily restraint was followed by the usual decline in adrenal ascorbic acid.

As a consequence he concludes that neither epinephrine, norepinephrine, histamine, nor acetylcholine is the sole factor in the hypothalamic-hypophyseal link, a conclusion with which most investigators would agree.

*Posterior lobe hormones.*—The view of Ranson and his colleagues that the cells secreting the posterior lobe hormones are situated in the posterior lobe of the pituitary and that their rate of secretion is regulated by nerve fibres from the supraoptic nuclei has been questioned by Scharrer & Scharrer (88). They propose that the nerve cells themselves secrete specific agents that pass along the axons of the nerves to the posterior lobe where they accumulate in the region of the capillaries. Scharrer (89), from anatomical considerations, has also suggested that one of these neuro-secretory products may be concerned with the release of ACTH, since he visualizes that in the median eminence the axons carrying the neuro-secretory material may make contact with the hypophyseal portal vessles. [See also Herlant (90).] While the concept of a neurosecretory material, formed in the cell bodies and passing along their axons to the site of its release into the blood stream, was first suggested in relation to the neurones innervating the posterior lobe, there is no reason to believe that it might not also be true for other hypothalamic nuclei.

There have been several investigations on the effects of posterior lobe hormones, particularly the antidiuretic (ADH) hormone, on anterior lobe function. Before considering these it is well to remember, as Pickford (91) has pointed out, that the rate of release of ADH is altered (a) by changes in the osmotic pressure of the blood, (b) by sensory reflexes, and (c) by activities in the central nervous system itself. Furthermore, acetylcholine has been shown to be an important transmitter of impulses to the supraoptic

nucleus, stimulating the release of ADH, but only if the cells of this nucleus are intact. Certain pharmacological agents such as morphine and ferritin also stimulate the release of ADH as judged by their effects on diuresis. On the other hand, epinephrine, whether injected or reflexly secreted, has been shown to be an inhibitor of ADH release. These effects should be borne in mind in considering the influence of ADH and other posterior lobe hormones on the anterior lobe.

Nagareda & Gaunt (92) found that while the injection of large amounts (400 mU) of vasopressin (Pitressin) into rats reduced the adrenal ascorbic acid smaller quantities (5 mU) had no effect. However, even the smaller amounts had a marked antidiuretic effect. Sobel *et al.* (93) report that in guinea pigs the intraperitoneal injection of five pressor units increased the output of urinary corticoids. They state that this is not due to a rise in blood pressure, release of epinephrine, or to a fall in serum sodium, but presumably to a stimulatory effect on the pituitary-adrenal axis. Sayers & Burks (94) find that the injection of 5 units of vasopressin, a very large amount, increases the blood level of ACTH in adrenalectomized rats.

Certain stimuli known to be associated with release of ACTH are now reported to also increase the secretion of ADH. Ginsberg (95) observed that hemorrhage is one such circumstance, while Mirsky, Stein & Paulisch (96) report that pain, noise, or containment in a strange environment increase the blood level of ADH of rats some threefold. They also state that similar increases occur in hypophysectomized rats, indicating that the source of the ADH must be in the central nervous system (97). They propose that vasopressin, oxytocin, or some closely allied substance may serve as hypothalamic "neuro hormones" responsible for activation of the anterior pituitary. Lloyd & Oldford (98), from studies of the hypothalamic content of ADH under various conditions, reach similar conclusions. Rothballer (99) using the Gomori staining method observed rapid depletion of this material in the hypothalamus, median eminence, and posterior lobe after exposure of animals to painful stimuli.

It would seem from the work of these investigators that various types of stimuli increase not only the rate of secretion of the hormones of the adrenal medulla but also those of the hypothalamico-posterior hypophyseal system. This parallelism also contains a paradox, as the former are stated to inhibit the rate of secretion of the latter, at least that of the antidiuretic factor. It should also be noted that while estimates of increased posterior lobe secretion have been made in terms of antidiuretic activity, it does not follow that ADH is necessarily the agent exciting ACTH secretion. It may be merely an indicator of the release of several substances, a point made by several investigators.

An entirely different interpretation of the role of posterior pituitary hormones in the regulation of ACTH secretion is offered by the work of Itoh & Arimura (100), Kimura (101), and Arimura (102). They observed that the fall in the adrenal ascorbic acid of rats given epinephrine or histamine, or

subjected to stress was largely prevented by (a) the prior injection of 20 mU of vasopressin; dehydration produced by thirsting; or by the intraperitoneal injection of 5 per cent glucose. Since these quantities of vasopressin did not in themselves influence the adrenal ascorbic acid they conclude that a posterior lobe principle, associated with vasopressin but not with oxytocin, inhibits the release of ACTH. They also state, in support of this view that dilution of the blood by the ingestion of a considerable quantity of water brings about a fall in adrenal ascorbic acid, an observation made previously by Nagareda & Gaunt (92) and Dexter & Stoner (103).

On the other hand it might be argued that epinephrine, either injected or secreted, has inhibited the release of ADH by its action on the hypothalamic centers, in consequence of which the rate of release of ACTH is increased. That this is not unlikely would appear from the work of Eversole & Giere (104) who find that while epinephrine and norepinephrine do not in themselves alter the ADH content of rat blood, they inhibit the usual rise in ADH that follows the injection of morphine. Further speculation is not justified since the effect of adrenal medullary hormones on diuresis is a complex one. These experiments with ADH do suggest, however, that alterations in the osmotic pressure of the blood may play an unexpected role in the regulation of ACTH.

A further example of a possible association between the supraoptic-hypophyseal system and ACTH is furnished by the studies of McCann & Brobeck (105). These investigators state that hypothalamic lesions which uniformly destroy a significant portion of this tract, not necessarily in the median eminence, block ACTH release. Their animals had diabetes insipidus in a varying degree. They state that the administration of large doses of vasopressin, but not oytocin (Pitocin), epinephrine, or small doses of vasopressin caused a fall in adrenal ascorbic acid. They believe that their results suggest that the release of ADH into the portal vessels is a factor in the regulation of ACTH secretion.

*Specific hypothalamic hormones.*—This term is used for agents which may serve as humoral transmitters to the anterior pituitary and which are not identical with any known hormone. They are believed to represent a specific agent of hypothalamic origin which may reach the hypophysis either through the systemic or portal hypophyseal circulation. Furthermore, such an agent is believed to have its origin in the nerve cells of one or more hypothalamic nuclei.

In 1952 Hellerstein *et al.* (106) reported that the daily injections of extracts of beef hypothalamus into infant rats was followed by a greater fall in circulating lymphocytes as compared to that of litter mates receiving extracts of beef medulla oblongata. Slusher & Roberts (107) now report that they have isolated two active fractions from beef hypothalamus. The first is water soluble and causes eosinopenia in rats but does not reduce the level of adrenal ascorbic acid. The second appears to be lipid or lipoprotein in nature. This decreased both eosinophiles and adrenal ascorbic acid and was obtained only from the posterior hypothalamus.

Guillemin (108) cultured anterior pituitary tissue from dogs and rats in roller tubes. Although good growth and excellent differentiation was obtained, ACTH could be detected in the nutrient medium only during the first 4 days of culture. None was found from the eighth to the twenty-second day. However, the addition of explants of posterior hypothalamus or median eminence to the culture on the fifteenth day led to the reappearance of ACTH. No effect was obtained by adding anterior hypothaiamus, brain cortex, spleen, or liver. The medium at the end of the culture period contained negligible amounts of histamine, epinephrine, norepinphrine, serotonin, vasopression, and oxytocin, nor did the addition of vasopressin reinstate ACTH formation. It is of interest that growth hormone was formed throughout the whole period of culture.

These elegant experiments, only briefly reported at present, would seem to offer an opportunity to study the *in vitro* effect of substances which have been proposed as affecting the secretory functions of the anterior pituitary. It will be noted in relation to the reports reviewed above on posterior lobe hormones that vasopressin did not stimulate ACTH release.

Saffran & Schally (109) have also used isolated systems. The one they have developed is more complicated than that of Guillemin. Anterior lobe tissue is incubated with the substance under examination as a possible transmitter, or with the tissue in which it is suspected it may be formed. After a suitable interval, the medium is removed and added to another vessel containing the quartered adrenal glands of rats. The quantity of adrenal steroids formed and released into the second medium is then determined. The results are expressed as units of ACTH activity. They report that epinephrine, norepinephrine, or hypothalamic tissue added alone to the flask containing anterior lobe tissue does not increase the output of ACTH. However, the addition of them together resulted in a threefold increase. Acetylcholine or serotonin cannot replace the catechol amines, but brain cortical tissue can substitute for hypothalamic tissue. The largest increases in ACTH were obtained when posterior pituitary and norepinephrine were added together (the effect of epinephrine, if any, is not stated). Posterior pituitary alone had no effect. Norepinephrine could be replaced by sphingosine, a substance also containing the secondary hydroxy amine grouping. The present interpretation of these experiments would be that both the catechol amines and a factor present in the hypothalamus and posterior lobe are necessary for ACTH release under these experimental conditions.

Another approach to this problem is that of Porter, Vanatta & Dillon (110) who removed the pituitary from dogs and then collected the blood draining from the severed stalk. When the plasma was injected into rats it brought about a reduction in the urinary excretion of sodium greater than that produced by comparable injections of blood from a systemic vessel. Injections of hypophyseal portal plasma for several days did not increase the weight of the adrenals of rats.

*Adrenal cortical steroids.*—The first suggestion as to the nature of the regulation of ACTH secretion was that it was controlled by the blood level

of adrenal steroids in such a way that a fall stimulated the secretion of ACTH while an increase depressed it. The experimental facts on which this is based are unchallenged so far as the effects of an increased blood level of adrenal steroids are concerned. For, as is well known, the administration of cortisone brings about an atrophy of the adrenal as severe as that produced by hypophysectomy and far in excess of that usually seen after stalk section or hypothalamic lesions. This atrophy does not occur if ACTH is given along with the adrenal steroids.

The effect of a fall in the blood level of adrenal steroids on ACTH secretion is seen at its height after total adrenalectomy where the blood level of ACTH increases many fold. The effect of smaller declines in blood adrenal steroids is not so clear. Sayers places much importance on the lowering in the blood level of the cortical hormone which he states may be brought about by an increased tissue utilization which is a consequence of many stressful conditions. The demonstration that significant decreases occur in the blood level of adrenal steroids with sufficient rapidity to account for ACTH secretion under many conditions has not been made. Sydnor & Sayers (111) subjected intact and adrenalectomized rats and dogs to the stress of laparotomy, or scalding. In the adrenalectomized animals the blood ACTH rose two to three times higher than in intact animals, a finding they attribute to the lower blood level of adrenal cortical hormones in these animals. Furthermore the high level of ACTH persisted much longer in them than in intact rats. Sydnor (112) subjected dogs to both unilateral and bilateral adrenalectomy and also found higher blood ACTH levels in the latter. To answer the criticism that changes in blood steroid level cannot occur fast enough to suppress a rapid release of ACTH, they report (113) that intravenous hydrocortisone can block the release of ACTH within 1 to 2 min.

Under more physiological circumstances, that is in exercising humans, Staeheln *et al.* (114) have found that the plasma cortical hormone level rises with the start of exercise, which is attributed to adrenal stimulation, then as exercise continues a significant fall below the resting level is found. In two cases of Addison's disease there was a more rapid removal of infused cortisone from the blood during exercise than at rest.

On the other hand, in adrenalectomized dogs subjected to trauma, Cowie, Ganong & Hume (115) found a greater rather than a smaller degree of eosinopenia in response to small doses of hydrocortisone given intravenously. They believe this indicates there is no increase in the utilization of hydrocortisone as a result of the trauma. Indeed, they consider that it may be less than in intact animals.

Bush (116) infused warm Locke's solution, or 5 per cent solution of albumen in saline, into one internal carotid artery of dogs, while occluding the other. The purpose was to acutely lower the level of adrenal cortical hormones in the blood perfusing the pituitary. This procedure caused a fall rather than a rise in the quantity of adrenal steroids in the blood of the adrenal vein. However, if cold solutions were infused the initial fall was fol-

lowed by a sharp rise. This increase did not occur if the splanchnic nerves were cut or if hydrocortisone was infused along with the solution. Although Bush concludes that a fall in the concentration of adrenal cortical hormones does not stimulate the release of ACTH, the results of experiments with the cold solutions could be interpreted as supporting the participation of epinephrine in the release of ACTH.

While the effects of acute changes in adrenal steroid levels may be still undetermined, there is no reason to doubt that their maintenance at abnormally high levels for a long period of time produces adrenal atrophy. The site of this effect of the adrenal steroids is of some importance. As mentioned above Ganong & Hume (25) concluded that they suppress ACTH secretion by a direct effect on the anterior lobe itself, a conclusion also supported by the studies of Kallman & Gordon (26). Furthermore, Keeler & Roberts (68) report that the prior administration of cortisone depresses the oxygen uptake and aerobic glycolysis of isolated anterior lobe tissue. Farrell & Laquer (117) gave very large doses of cortisone to dogs (100 mg. a day) for as long as 71 days. At the end of this time they found the ACTH content of the pituitary reduced to 2 per cent of its initial value. They argue that cortisone not only blocks the release but also the synthesis of ACTH, since, if only the former occurred, an accumulation of ACTH in the gland would be expected to occur.

The site of action of cortisone has a bearing on the hypothesis that the hypothalamic nuclei are important in ACTH secretion. If it could be shown that the adrenal steroids depressed the activity of these centers, and thus reduced the release of a transmitter of hypothalamic origin, then the view that all conditions, including a changing level of adrenal steroids in the blood, are mediated by the hypothalamic-hypophyseal portal system would be greatly strenghened.

One approach to this problem has been to study the effects of pharmacological agents that are known to depress hypothalamic activity. It should of course be realized that other areas of the central nervous system may be equally affected by such agents. The two that have been investigated in this regard are morphine and the barbiturates. Briggs & Munson (118) found that morphine given alone to rats reduces the adrenal ascorbic acid. However, if given to rats previously anesthetized with pentobarbital, no depression of adrenal ascorbic acid was observed. Such animals show no fall in adrenal ascorbic acid when subsequently given histamine, epinephrine or vasopressin, nor is any seen after laparotomy or unilateral adrenalectomy.

If the rats are adapted to morphine by daily injection of the drug for 4 days or more, no change in adrenal ascorbic acid can be elicited by any of these procedures and preliminary anesthesia with pentobarbital can be omitted. Ohler & Sevy (53) also report that morphine prevents the fall in adrenal ascorbic acid after unilateral adrenalectomy. They also find that while adrenal cortical extract had some inhibitory effect on this response, Dibenzyline had no effect. They contend that a decreased level of blood corticoids is

a basic factor in the regulation of ACTH and that morphine blocks the response to this either at the pituitary or hypothalamic level.

The barbiturates also modify the response of the adrenal ascorbic acid to certain circumstances and since they are believed to depress both the hypothalamus and other autonomic ganglia this effect is of some interest. Ronzoni (119) observed that rats deeply anesthetized with pentobarbital did not respond to cold exposure with an increase in blood glucose or a fall in adrenal ascorbic acid. Exposure to heat or hemorrhage still produced a fall in the adrenal ascorbic acid without increasing the blood glucose. Consequently, she concludes that secretion of medullary epinephrine is not essential for ACTH release. Cronheim & Hyder (120) now find that the effect of salicylic acid on the adrenal ascorbic acid of rats is abolished by deep anesthesia with pentobarbital. They suggest that salicylic acid stimulates the hypothalamic centers associated with ACTH secretion.

## Miscellaneous

Weisz and his colleagues (121, 122, 123), observed that 24 hr. after high spinal section in rats unilateral adrenalectomy no longer led to a fall in adrenal ascorbic acid. They suggest the use of such animals for the assay of ACTH. They also report that in these animals there is a delayed response to epinephrine and that after unilateral adrenalectomy there is also a delay in the hypertrophy of the remaining gland. These experiments are of interest since they show that (*a*) prevention of the passage of nerve impulses to the central nervous system does alter the character of the response to stress although (*b*) the response to epinephrine injection, albeit delayed, still occurs, as does hypertrophy of the adrenal.

Jailer (124) had previously reported that the adrenal ascorbic acid of infant rats does not decline after the injection of epinephrine, a fact attributed to the absence of myelination of essential hypothalamic pathways. Thompson & Blount (125) and Rinfret & Hane (126) now report that they cannot confirm these observations since the former found a good response to epinephrine at 1 day after birth, while the latter observed that pituitaries of infant rats contain adequate amounts of ACTH.

Langley, Scokel & Moore (127) report that the falls in eosinophiles, and adrenal ascorbic acid and cholesterol of rats exposed to cold were lessened when the carbon dioxide content of the atmosphere was kept at 5 per cent.

Khalil (128) compared the degree of adrenal atrophy in hypophysectomized rats with that in rats receiving large amounts of sodium ascorbate by vein each day. He states that the adrenal atrophy is almost completely prevented by such treatment.

Leach, Heath & Strohmeyer (129) stimulated, by means of permanently implanted electrodes, the septal area of cats for 20 min. each day for several days. They report that the urinary excretion of 17-ketosteroids was increased by more than 100 per cent, and in adrenalectomized animals even greater responses were obtained.

Maren (130) studied the hypothalamus in two cases of Cushing's syndrome associated with bilateral adrenal hyperplasia. He could observe no differences between them and those of a control group. He also could observe no change in the hypothalamus of genetically dwarfed mice, a condition where the anterior pituitary is virtually absent.

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# REPRODUCTION[1,2]

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Papers dealing with or incidentally contributive to the physiology of reproduction are being added to the scientific literature at an average rate of 10 or more per week. These have brought additions to our knowledge in the past year as well as many reminders of the host of important matters that await solution. Investigative work in this field has in recent years turned more and more toward the practical application of present knowledge, particularly with respect to breeding and rearing of economically important domestic animals and matters of fertility and sterility in man.

Several useful volumes have appeared during the year. Of broad interest and significance is the May, 1955 issue of the *British Medical Bulletin* comprised of 17 articles on the general topic "Hormones in Reproduction" (1). In scope, these cover fifty years of development in the field of reproductive endocrinology. Since it totals only 81 pages, it is not surprising that there are omissions in this accounting of a field which has, as Parkes, the editor, states, "blossomed so profusely in so short a time." "The fruit," Parkes adds, "is now before us as an impressive body of knowledge festooned, as might be expected, with loose ends of observation and deduction." This issue represents a significant statement of progress to date. Other valuable volumes include the proceedings of the first Josiah Macy Jr. Foundation Conference on Gestation (2), the Cold Spring Harbor Symposium on The Mammalian Fetus (3), the National Research Council Conference on Pregnancy Wastage (4), and the 1953 Laurentian Hormone Conference (5). Folley (6) reported briefly on the conference on Comparative Endocrinology of the Vertebrates, the proceedings of which are to appear subsequently. Hammond reviewed "Light Regulation of Hormone Secretion: Reproduction" (7).

## HYPOPHYSIS AND GONADOTROPHINS

Some further purification of the follicle stimulating hormone is reported by McShan & Meyer (8), using anion exchange resins, and by Steelman *et al.* (9), using fractionation with ethanol and digestion with pancreatin. Though of high biological purity, neither FSH preparation is pure by physicochemical criteria. The extraction of pituitary gonadotrophins from urine has been greatly facilitated by adsorption on aluminum hydroxide (10) and on collodion filters (11). In the later study the urine was simply shaken with the filters for 2 to 3 hr.

[1] The survey of the literature for this review was completed at the end of June, 1955. At that time, the June issues of some journals, particularly the foreign ones, had not yet become available.

[2] The following abbreviations are used throughout this chapter: HCG (human chorionic gonadotrophin); PMS (pregnant mare serum); FSH (follicle stimulating hormone); LH (luteinizing hormone); DCA (deoxycorticosterone acetate); ACTH (corticotrophin); PAS (periodic acid-Schiff).

Paesi and associates (12), using a modified assay procedure, aver that following ovariectomy the FSH content of rat pituitary glands is increased fivefold and, contrary to the weight of existing data, suggest that in males no such change follows gonadectomy. These FSH values were reduced by estradiol in all but the intact female rat. Pituitary and urinary gonadotrophins assayed in guinea pigs are reported to exhibit a constant FSH to LH ratio of activity of 5:1 (13). In immature rats given cortisone, the gonadotrophic potency of the pituitary glands is accentuated and the ovaries and uteri of the injected animals are generally enlarged (14).

In the human hypophysis no correlations could be established between cell picture using PAS staining and gonadotrophic hormone content [Burt & Velardo (15)]. Wilson & Ezrin (16) identify three types of chromophilic cells in the human hypophysis, two being PAS-positive; the latter correspond to the gonadotrophs and thyrotrophs in rat hypophysis. Purves & Griesbach (17) now recognize in rat hypophysis a peripheral basophil that is FSH-producing and a more centrally located basophilic cell that is responsible for secretion of luteinizing hormone. Progressive changes in the preponderance of these two types of basophils parallel closely the short- and long-term qualitative changes in gonadotrophic hormone content after gonadectomy (18). Lockhart & Finerty (19) substantiate the existence of two kinds of basophils: thyrotrophs centrally placed, and gonadotrophs located peripherally. However, the latter are found throughout the anterior lobe after castration; this condition is reversed by estrogens. The mouse pars distalis has but acidophils and amphophils, and these show marked cyclic, reciprocal alterations in number (20). Mitotic activity in the anterior lobe of the pregnant mouse increases in the second half of gestation, accounting perhaps for the increased weight of the gland and possibly reflecting an increased level of circulating estrogens (21). The induction of pituitary tumors in mice with estrogen is dependent upon and accentuated by thyroid hormone (22).

## REPRODUCTION IN THE FEMALE

Intra-ocular ovarian grafts in rabbits took better and were stimulated to enlarged size with FSH (23) and responded to HCG like ovaries *in situ*. Though ovaries grafted into the spleen in castrated immature rats enlarged thirty- to fortyfold, vaginal diestrous was sustained, and the uterus showed but slight growth (24). An extensive study of the hormonal activity of rat placenta [Ray *et al.* (25)] has confirmed the presence of luteotrophic activity and shown that this substance (or substances) also exhibits mammogenic, lactogenic, and, to a weak extent, crop sac-stimulating activity. Autotransplants of rat anterior hypophysis to the renal capsule showed no functional impairment of their luteotrophic function in respect to deciduoma formation [Everett (26)]. That this disruption of the normal relationship of hypophysis and hypothalamus did not interfere with hypophysial function has significance in the disputed importance of hypothalmico-hypophysial relationships.

*Ovary.*—Since the ascidians are generally believed to occupy a key position in the evolution of the Chordata, their reproductive organs and functions

are of unusual interest. In *Ciona intestinalis* details of ovarian structure, origin of oocytes and ovarian cycles are added by Pérès (27). He found no formation that might be compared to corpora lutea disputing Carlisle's earlier claim that such exist. The corpus luteum present during delayed implantation in the armadillo was found to be functional, hence the delay in implantation in this species is more likely to be attributable to some improper ratio of estrogen and progestin than to an insufficiency of progesterone [Talmage (28)]. Amoroso (29) describes fetal giraffe ovaries showing large follicles and "extensive luteinization." Along this same line Parkes (30) has referred to the oddity that seal pups are seemingly born pseudopregnant.

The distribution and concentration of proteins are quite similar in the fluids of normal follicles and ovarian cysts and differ from normal human serum only in having a reduced globulin component [Perloff *et al.* (31)]: Ovaries of young rats have been frozen to −79°C., thawed, implanted, and shown to form functional grafts even after storage in the frozen state for periods up to one year [Deanesly (32), Parkes & Smith (33)]; the physiological significance of such experiments, while of considerable interest, remains to be determined. The young offspring born of mice in which the ovaries have once been painted with methylcholanthrene exhibit localized hemorrhagic areas over the body (34). The effect persists in subsequent litters as well. The ovarian hyperemia reaction to HCG in immature rats is affected by the level of adrenal cortical function (35). Additional information on the localization of histochemically demonstrable lipids, mucoproteins, and glycoproteins in human ovaries (36) and phosphatases in rat ovaries (37, 38) is at hand. On the basis of a new tinctorial reaction it is claimed that the secretion of estrogen (probably estriol) by the corpus luteum can be demonstrated (39).

Female mice exposed to 4 $\mu$c. $I^{131}$ or 10 r x-ray weekly showed a reduction in fertility, anovular ovarian cortices and early evidence of neoplasia. The same doses did not affect male mice [Rugh (40)]. Hybrid mice given 300 to 500 r to the ovaries or 100 to 400 r to the whole body produced but one litter and were sterile thereafter (41). The incidence of ovarian tumors was 100 per cent. It appears that activation of the pituitary-ovarian axis was achieved in rabbits by applying 800 r to the whole body or by exposing only the pituitary region to irradiation; the intra-ocular endometrial implants which they bore were seen to respond within 24 to 48 hr. by a significant increase in area (42).

*Ova.*—Living human tubular and follicular ova have been cultured *in vitro*, [Duryee (43); Shettles (44)] fertilized, and observed to reach the eight-cell stage in approximately 60 hours. Denudation of such ova has been achieved by aid of fibrinolytic, enzymatic activity of the tubal mucosa (45). Hertig *et al.* (46) give the cell number and age of four fertilized and normal appearing human ova as the following: 2-cell, 26 hr.; 12-cell, 72 hr. 58-cell, 96 hr.; 107-cell, 108 hr. They incline to the view that abnormalities may arise through defects that are intrinsic to the ovum, such as a delay in the segmentation rate, rather than to an unfavorable environment. An opposite view is expressed by Hughes *et al.* (47) who emphasize the role of the endometrium in fetal wastage.

Colchicine, hot-shock treatment, and ether anesthesia have been used to induce heteroploidy in early mouse embryos (48, 49). Delayed fertilization (50) and colchicine treatment of semen (51), contrary to previous suggestions, has been ruled out as a factor in polyspermia and triploidy. Lysenko and his school are reported (52) to hold the view that polyspermy is a normal and advantageous feature in fertilization. Austin (53) has succeeded in increasing the incidence of polyspermia in hooded rats to 20 per cent by artificial hyperthermia after delayed mating. A recent study of rat and mouse eggs, however, reaffirms that the penetrability of the zona pellucida to spermatozoa decreases speedily after entry of the first spermatozoön [Braden *et al.* (54)]; a second spermatozoön in the opposite hemisphere of the ovum is sometimes seen.

After gonad stimulation in rabbits, radioactive phosphorus is seen to concentrate in the theca interna and granulosa, whereas radioactive sulfur gathers in the cumulus oophorus (55). The fertility of mice given 60 $\mu$c. $S^{35}$ per gm. of body weight and subsequently mated was not impaired nor the number of embryos per pregnancy reduced (56). Rabbit ova, transferred after cooling to the fallopian tubes of recipient does, underwent parthenogenetic development to the blastocyst stage when examined at 6 days [Chang (57)]. These were diploid in chromosome number because of failure of second polar body emission. Olsen & Marsden (58) report the occurrence of natural parthenogenesis in turkey eggs.

*Ovulation.*—Ishizuka *et al.* (59) state that they have succeeded in advancing time of ovulation in a series of normal women by the subcutaneous injection of over 3000 I.U. HCG daily during the second to seventh days of the menstrual cycle. They base their finding on several criteria for ovulation including thermal shift, pregnanediol in the urine, and changes in the endometrium. The cycles were shortened accordingly. Detection of the time of ovulation in women by serial vaginal smears has been under thorough study, with inconclusive (60) findings being reported. Bastian (61) has succeeded in blocking ovulation in the normal hens with atropine and the adrenalytic agent, SKF 501. The effective dosages, however, are great and he urges caution in attributing the action to blockade of cholinergic and adrenergic components in the mechanism controlling ovulation. In this connection Fraps & Conner (62) obtained evidence that ovulation-inhibiting agents may act on processes that precede pituitary activation for release of ovulating hormone, i.e., on processes concerned with follicle ripening. In the 43.5 hr. interval between the last ovulation of one clutch and the first ovulation of the succeeding one they could block ovulation in a high percentage of hens by giving the usual blocking agents as much as 38 hr. prior to expected ovulation. In the fowl the "spontaneous" neurogenic stimulation of the pituitary and the release of ovulating hormone appear to occur simultaneously, to be of short duration, and to precede ovulation by about 8 to 14 hr. (63, 64).

With respect to the secretion of ovulatory hormone, reserpine has been added to the list of substances which block ovulation in rats (65). Lesions

in the olfactory bulb have been shown to prevent histamine-induced ovulation in rabbits, suggesting an importance of rhinencephalic pathways for the initiation of ovulatory process [Sawyer (66)]. By means of electroencephalographic recordings taken from various regions of the brain in female rats at various stages of the estrous cycle, Critchlow & Sawyer (67) discovered marked localized excitations in the preoptic area and hypothalamus within the critical 2 to 4 P.M. period preceding ovulation. Thus, a tangible clue to the central nervous stimulatory process that leads to activation of the anterior pituitary and ovulation may have been provided.

*Estrus and estrous cycles.*—Hafez (68) has timed the events in the estrous cycle in buffalo cows. Ovariectomized ewes treated with estrogen seldom exhibited normal estrous behavior, but, by interspersing periods of progesterone treatment (75 mg. in six twice daily injections) followed in two days by a switchback to estrogen treatment, recurrent estrus with typical estrous behavior was seen in all ewes (69, 70). The insertion of beads into the ovine uterus in the early luteal phase shortens the mean cycle length by three days whereas the same maneuver in late luteal phase extends the cycle by seven days. Since these changes are cancelled by resection of the uterine section containing the beads [Nalbandov *et al.* (71)], it is assumed that a neurogenic mechanism is involved. The presence of rams has been shown to have a favorable and hastening influence on ovarian activity and estrus in ewes (72, 73).

There is considerable current disagreement as to the essentiality of an intact hypophysial portal system in the regulation of the rhythmic functions of the anterior pituitary. The dispute happens to hinge rather critically at the moment on observations concerning the induction of estrus in stalk-transected ferrets by light stimulation. Zuckerman (74) reports that three operated ferrets, in which no vascular link was demonstrated between the region of the median eminence and the pars distalis, came into estrus after exposure to added lighting in precisely the same manner as unoperated controls. Donovan & Harris (75, 76) severed the stalk in 20 ferrets placing a waxed paper barrier between the cut ends of the stalk in some animals. Approximately one-half of the operated ferrets responded to light, while the remaining ones failed to do so. Zuckerman's data need to be extended, and the data of Donovan & Harris seem by no means decisive. The possibility has again been raised that peripheral nervous pathways may be involved [Abrams *et al.* (77)]. Sixteen female ferrets were subjected to bilateral superior sympathetic ganglionectomy and exposed to added illumination. All remained anestrous, whereas 14 intact controls maintained under the same condition reached full estrus. That the effect of light does not operate via conversion of estrone in the skin to the more active estradiol 17 $\beta$ was ascertained by Repke & Markwardt (78). Obese mice have been noted to exhibit a constant estrus which, unlike other occurrences of this phenomenon, is attributable to an extreme abbreviation of the cycle resulting in consecutive periods of estrus. It is caused by an excessive release of FSH and LH and can be interrupted by testosterone or pentothal but not by progesterone (79). Fels (80) reports that ligature of the oviduct in rats produces a state of prolonged or

permanent estrus. The mechanism responsible for this interesting observation merits further study.

*Menstrual cycle.*—The length of the menstrual cycle has been studied in large groups of women in an attempt to identify possible patterns of variation and the factors which influence variation [Collett *et al.* (81); McKeown *et al.* (82)]. These data indicate that length of cycle is most constant at 25 to 34 years of age, less at 20 to 24 and 35 to 39, and least at 17 to 19 and 40 to 50 years. Mean cycle length decreases with age and is not affected by duration of lactation. The luteal phase lasts 12 to 15 days and varies at all ages but is more constant than the follicular phase. The incidence of anovulatory cycles is highest at 17 to 19 years, moderate at 40 to 50 and least at 25 to 34 years. Though disturbances in thyroid function unquestionably may affect menstrual histories in certain patients and not in others, Buxton & Herrmann (83) in a well-controlled study found that thyroid administration was of no value in euthyroid patients complaining of sterility and menstrual disorders. Swyer (84) also affirms that the indiscriminate use of thyroid "in large or in physiologically insignificant doses is wholly to be deplored." The cyclic aspects of the urinary secretion of estrogen have again been analyzed in terms of estriol, estrone, and estradiol-17 $\beta$ [Brown (85)]. Continual determinations of Hooker-Forbes activity in the serum of women with normal cyclic function show progesterone appearing in the serum during the follicular phase and rising markedly just prior to ovulation (86). Starting with an estrogen- or progesterone-withdrawal menses [Krohn (87)] recurrent bleeding at eight-day intervals can be maintained in spayed monkeys with suitably spaced series of progesterone injections. Marked changes in plasma volume and protein mass occurring rhythmically during the menstrual cycle in baboons can be related to the perineal swelling and involution [Cohen (88)].

*Human chorionic gonadotrophin.*—Simplifications in the method of extracting HCG from pregnancy urine for testing purposes have been introduced (89, 90). Studying HCG preparations of high potency by zone electrophoresis on starch, Raacke, Li & Lostroh (91) identified two components that differ somewhat in biologic potency and isoelectric point. Drescher (92) claims to have fractioned an FSH component from HCG. HCG was found to predominate in the mucoprotein fraction isolated from early pregnancy urine but not in that of normal male urine (93); the carbohydrate content of purified HCG and urinary mucoprotein is strikingly similar. Alpha particles inactivate HCG (94). Pilocarpine and ephedrine strengthen the hormonal effect of HCG in toads and mice, whereas atropine and dihydroergotamine diminish it (95), suggesting autonomic modulation of gonadal sensitivity. The response of puberal rats to HCG was found to be accentuated by hypothyroidism (96) and to be unaffected by adrenalectomy (104). Pregnant mice given HCG are reported to develop pituitary lesions mimetic of those seen in Sheehan's syndrome in man (97). Slow disposal of the hormone was suggested by a study of its rate of disappearance in the blood of men following injection of large doses and by the fact that it is not augmented by using polyvinylpyrrolidone as the injection medium (98). HCG dissolved in serum

is not potentiated over that in saline when assayed in male rats, but, by the uterine-weight test, the apparent potency is increased twofold or more (98, 99). Loraine & Brown (100) and Diczfalusy (101) have continued to refine the bioassay of HCG. Renal excretion of HCG during pregnancy is thought to be constant and approximates 32.5 per cent of the blood content per day, indicating a turnover of hormone in the blood four times daily (102). Very large single doses of HCG given to castrated and noncastrated women increased the excretion of neutral 17-ketosteroids (103).

*Pregnant mare serum.*—The ovarian response to PMS in both adult and immature rats was reduced by adrenalectomy and was restored by cortisone but not by DCA [Mandl (104)]. The ovaries of immature mice given single injections of PMS in doses of 1.1 μg. or more show enlargement and luteinization; with lesser doses, even though these ovarian changes completely disappear, the uterus may continue to show responses indicative of estrogen and progesterone stimulation [Green (105)]. Hill (106) and Wallace (107) attest to the usefulness of PMS in improving the fertility of cows and ewes respectively. For best results in ewes 650 to 1000 I.U. should be injected on the twelfth, thirteenth, or fourteenth day of the estrous cycle. Proneness to abnormal development is not increased in PMS-ripened ova. Varicak (108) correlated blood levels of mare serum hormone with a patchy sloughing of the uterine mucosa forming "craters" between the fifth and twenty-second week of gestation.

*Antifertility factors.*—Among several species of plants screened for *in vitro* antigonadotrophic activity, three species of *Lithospermum*, the red raspberry, *Rubus idaeus*, and a species of Borage, *Borago officinales*, had appreciable activity (109). Mediation of the well-known inhibiting effect of *Lithospermum ruderale* on testis weight requires the presence of the adrenal gland (110). The drug Enheptin (2-amino-5-nitrothiazole), which inhibits sexual manifestations in domestic fowl, was shown (111) to reduce gonadotrophic potency in the hypophysis of capons and cockerels. It does not interfere with the testis response to exogenous gonadotrophins. An extract of pineal gland produced a significant retardation of ovarian development in immature rats (112).

*Estrogens.*—Quantitative aspects of the bio-assay of estrogens in various hybrid mice and the related importance of certain biological factors have been dealt with in a series of papers from the laboratory of Biggers & Claringbold (113 to 118). Dorfman & Dorfman (119) utilize for the same purpose the uterine weight of the immature rat and provide a statistical analysis of the assay. Braunsberg and associates (120, 121) have introduced a modification of the fluorometric method of estimating estrogens in body fluids and also described a method for the separation of estrone, estradiol, and estriol in such fluids by partition chromatography. The relative effectiveness of these three estrogens in promoting uterine growth in rats when administered separately and in various combinations has been determined by Hisaw *et al.* (122). Near maximal uterine enlargement was attained by 72 hr. On concurrent administration, estriol, known to be least active of these three estrogens, brought about a reduction of the growth promoting action of estradiol

or estrone. That reproductive function may be badly impaired by estrogens given in early life has been confirmed by Braden (123). Persistent infertility, which was referable in most animals to a failure to ovulate, has been produced in rats and mice by estrogen given over a four-week period beginning shortly after birth.

Estrogen recovery experiments performed in cattle showed a tremendous reduction in blood level within 30 min. (124) following an intravenous injection of 10 mg. estradiol 17 $\beta$. Only 31 $\mu$g. appeared in the urine in 24 hr., leaving the surmise that much must have been eliminated via fecal excretion. The pattern of excretion of estrone 16-$C^{14}$ and estradiol-17 $\beta$-16-$C^{14}$ in women, quite to the contrary, showed that as much as 65 per cent of the injected dose was recovered in the urine and that fecal excretion was minor [Beer & Gallagher (125, 126)]. The urinary recovery following administration of deuterium-labelled estrone to pregnant subjects was, however, very low (127). The saliva of both male and female patients contained demonstrable estrogenic activity following the intravenous injection of 1 gm. stilbestrol potassium sulfate (128). The excretion pattern in rabbits has been determined for a series of synthetic estrogens [Jellinck (129)]. These compounds do not yield estrone, estradiol, or estriol in the urine. Ethinyl estradiol and triphenylchlorethylene were recovered unchanged in the feces. Rat liver homogenates possess an enzyme system which binds a metabolite of estradiol-16-$C^{14}$ to protein [Riegel & Mueller (130)], and the radioactivity is not freed from the protein by cleavage of disulfide, glycosidic, or ester linkages. The conversion of estrone to estradiol by human and rabbit red blood cells has been demonstrated by means of paper chromatography and bioassay of eluted spots (131). From a study of the metabolic fate of deuterium-labelled estrone, Pearlman (132) was able to conclude that progesterone does not influence the course of estrogen metabolism, contrary to a long standing observation.

Further studies have been made of the estrogenic substances found in plants, particularly those that are consumed by farm animals. It is a fair assumption that the sterility seen in sheep in certain areas of Australia is attributable to genistin, the estrogenically active glucoside of 4′,5,7-trihydroxyisoflavone, present in certain species of subterranean clover. Other related isoflavones and their derivatives are under investigation [Cheng *et al.* (133); Bradbury & White (134)]. The estrogenic potency of genistin is very low (135, 136); hence it may more properly be described as a proestrogen. Mice grow and reproduce normally when genistin is added to the diet (137).

A 10 to 15 min. daily bath in the mud of the St. Moritz Spa is reported to elicit an estrous reaction in spayed rats and to prompt the return of estrous cycles in old anestrous females (138). Kislak & Beach (139) have shown that the aggressiveness which the female hamster displays in the diestrum or after ovariectomy is somewhat exacerbated by estrogen but is completely inhibited by an estrus-inducing dose of progesterone. The ovarian hormone which renders the female receptive also abolishes her tendency to attack the

male. The estrogens have been reviewed anew in terms of their biology (140), biochemistry (141), bioassay (142), distribution in plants (134), and related synthetic products (143).

*Progesterone.*—From a study of the effect of threshold amounts of estrogen and progesterone on vaginal changes in the rat, Ford (144) concluded that progesterone does not participate in the cornification reaction. Single injections of 5 to 50 mg. progesterone delayed estrus in rats for 10 to 19 days (the effectiveness being proportional to the dose), and 5 mg. twice weekly postponed estrus indefinitely [Slechta *et al.* (145)]. By comparison, methyl progesterone and ethinyl testosterone were respectively less effective and ineffective. No adverse effects on subsequent reproductive function were seen in any of these experiments. However, when heat was inhibited in gilts by treatment with 25 or 100 mg. progesterone [Baker *et al.* (146)] from the tenth or fifteenth day through the twenty-eighth day of the cycle, the subsequent heat was characterized by more cysts, fewer ovulated ova, and greatly reduced fertility. In a similar vein, Alloiteau (147) maintains that the interruption of permanent estrus by the administration of progesterone to rats with hypothalamic lesions is the result of an inhibition of FSH release by the pituitary gland.

Using infrared spectroscopy, Zander (148) has made a chemical identification of progesterone in human blood. The average concentration in plasma during the second half of pregnancy is given as 0.142 μg. per ml. (Forbes' previous value by biological test is 2.0 μg. per ml.). The half life of progesterone in the blood stream may be quite brief. Following the intravenous administration of 200 mg. progesterone to ovariectomized women, Zander (148) found only 2.540 μg. progesterone per ml. plasma at 3 to 5 min., 0.197 μg. at 2 hr., and none at 24 hr. Zarrow *et al.* (149) also found that 90 per cent of the activity had disappeared 10 min. following an intravenous dose of progesterone in rabbits; the loss was only a little less severe in the absence of the liver or kidneys. From determinations of the progesterone content of the tissues, especially fat, these authors were able to conclude that the principal factor in the rapid loss of progesterone from the blood stream is diffusion to the tissues. The Hooker-Forbes test has been used to follow the blood level of progesterone throughout the menstrual cycle in women (150), in ewes through the estrous cycle and through pregnancy (151), in rabbits during pregnancy and the puerperium (152), and in pregnant ovoviviparous snakes (153). A slightly greater Hooker-Forbes activity in the umbilical artery over the umbilical vein in human and goat pregnancies at term suggests a source of progesterone in the fetus [Forbes (154), Hoffman & Uhde (155)]; with good evidence that the placenta secretes progesterone, it is surprising that these levels are not reversed. Progesterone-like activity has previously been detected in the blood of fowl, and now a new role has been ascribed to progesterone: activation of the feather papilla [Shaffner (156)].

The earlier data of Somerville & Marrian indicating that the uterus was of importance in the metabolism of progesterone has been corrected in the light of new data by others including Marrian *et al.* (157) who showed that

the recovery of pregnanediol from progesterone-injected male and female subjects is in no way related to the presence or absence of the uterus. Using similar methods but employing patients that had been both bilaterally ovariectomized and adrenalectomized, Allen *et al.* (158) obtained convincing evidence that neither of these organs share significantly in the breakdown of exogenous progesterone. Modifications and inovations in the methods of determining urinary pregnanediol have been reported (159, 160). Increases in progestin activity as judged by the Hooker-Forbes test have been noted in the blood of rats and rabbits following administration of DCA [Lazo-Wasem & Zarrow (161)]; the principal sites of this conversion were found to be the adrenal glands and kidneys. The same workers (162) noted a release of progesterone-like activity from the adrenal cortex following stimulation by corticotrophin *in vivo* or *in vitro*, but not by HCG. The bio-oxygenation of progesterone can be accomplished by fermentation with fungi of the class Basidiomycetes (mushrooms) [Roland & Weiner (163)].

*Relaxin.*—Measurement of the relaxin content of the blood of pregnant women reveals levels equivalent to 0.2 guinea pig units per ml. of serum at 7 to 10 weeks, increasing to 2 guinea pig units at 38 to 42 weeks and falling to zero within 24 hours after delivery (164). The focus of interest in relaxin has shifted to its reported ability to abate uterine contractions in threatened premature labor [Abramson & Reid (165); Majewski & Jennings (166)]. Much additional work is required to fully assess its usefulness for this purpose. Crelin (167) reports that transplanted pelves in male and female mice failed to respond to estrogen and relaxin but those of the castrated male and female hosts exhibited characteristic replacement of the symphysial cartilage with an interpubic ligament, resorption of the medial ends of the pubes, and development of a gravid-like flexibility of the sacroiliac joints. Additional studies include the local placement of estrone particles over the symphysis (168) and a description of the histochemical changes taking place in the interpubic gap during and after pregnancy in mice (169). Principal among these is the loss of metachromasia, basophilia, and PAS-staining that heralds the breakdown of the cartilage in the last week of gestation; it is replaced first by fine argyrophilic fibers and later by collagenous connective tissue.

## Female Reproductive Tract

*Uterus.*—Abrahams & Pickford (170) have been able to make simultaneous observations on urine flow and spontaneous uterine motility in conscious dogs. Stimuli such as emotion, or intravenous injection of NaCl, acetylcholine, or sucrose solutions which inhibited water diuresis also enhanced uterine contractility which was "co-terminus with the antidiuresis." Contrariwise, when diabetes insipidus was present, uterine activity was low or nil. These results are believed to indicate a simultaneous release of the vasopressor and oxytocic factors by the neurohypophysis, but it is emphasized that the data also support those results of previous workers who have found a considerable disproportion in the rates at which these two factors are released despite a constant intraglandular ratio of 1:1. In rats, a dose of

oxytocin which just barely increases uterine activity during metestrus causes a threefold increase during proestrus [Genell (171)]. Sawyer (172) has shown that the oxytocinase activity in homogenates of rat uteri increases strikingly during pregnancy. In sheep, spontaneous uterine activity and sensitivity to pitocin and adrenaline is low until late pregnancy. Subsequent increases in these parameters however may exceed the nonpregnant level (173).

Estrogen is well known to effect an increase in uterine glycogen in ovariectomized rats. This is quickly reduced by intraperitoneal administration of epinephrine or posterior pituitary preparations [Kostyo & Leonard (174)]. Contrary to previous reports, the administration of estrogen to ovariectomized rats failed to effect an increase in the rate of glucose uptake by the isolated uterus [Hopkinson & Kerly (175)]. Under the same conditions progesterone accelerates the rate of glucose uptake.

The contractile material of uterine muscle responds like actomyosin to adenosinetriphosphate but differs from it in physicochemical properties and has been named actotropomyosin. The amount of actotropomyosin in the nongravid uterus is low while that in gravid corpus uteri is high [Naeslund & Snellman (176)]. The kinetic, tensiometric, and electrical characteristics of uterine muscle have been studied by Csapo & Goodall (177), Schofield (178), and Steer (179).

Recent studies on the cervix have emphasized the rhythmic changes in the quantity and viscosity of cervical mucus and its property of arborization, i.e., on desiccation of thin films of mucus, leaf-like patterns of crystallization develop [Zondek (180, 181)]. Crystallization is attributable to electrolytes in the mucus. The fact that only the mucus secreted some days before and immediately after ovulation crystallizes leads to the supposition that estrogen promotes and progesterone inhibits crystallization [Urdan & Kurzon (182)]. The limits of the usefulness of this new observational tool have not been established. Wheeler & Danziger (183) were unable to identify any cyclic changes in the human cervix using a wide variety of histochemical techniques.

*Vagina.*—There is likewise little cyclic variation in the histochemically demonstrable alkaline phosphatase in human vaginal epithelium according to Lang *et al.* (184). In rat vagina sulfhydryl groups were found in the basal layers of the epithelium during diestrum, and these shifted in proestrous to the layer of squamous transformation where disulfide groups also appear, thus lending support to the belief that —SH groups are converted to —SS groups in the process of keratinization (185). Explants of vaginal epithelium from infantile rats reach the keratinized state more rapidly when estrogen is added to the culture medium than without it (186, 187). The lack of vitamin A appears to be a factor in these experiments since addition of this vitamin to the culture medium prevents keratinization in the nonestrogenized controls and delays its appearance when estrogen is present. The sensitivity of the vagina of the ovariectomized rat to estrogen has been found to exceed that of the uterus by a factor of approximately 30 (188).

*Pregnancy.*—Implantation of blastocysts in mice artificially ovulated and

induced to mate prepuberally is a rare occurrence [Runner & Gates (189)]. They will implant, however, if transferred to a properly prepared mature host. The high incidence of microscopic or macroscopic bleeding in early pregnancy in women is believed to have physiological significance [Speert & Guttmacher (190)]. It has been noted that mice injected with DCA in early pregnancy abort their young (191). Pregnant women of Nagasaki showing major signs of irradiation injury evidenced a fetal morbidity and mortality of 60 per cent whereas for those with lesser exposures to radiation the figure was 10 per cent as compared with 6 per cent in nonexposed controls (192).

The African elephant, somewhat like the horse, has been found to replace its corpora lutea of gestation at mid-pregnancy by the luteinization, without ovulation, of graafian follicles [Perry (193)]. Smith (194) has reported that among 20 pregnant rhesus monkeys completely hypophysectomized at times varying from the twenty-seventh to the one-hundred and twenty-sixth day of gestation 10 went to term and six living babies were delivered (two by Caesarian section). The gestation period was normal, the parturition exhausting, and lactation brief. In a subsequent paper Smith (195) showed that the adrenal cortex in the pregnant hypophysectomized monkey did not atrophy so long as the placenta was present. Schmidt & Hoffman (196) gave large amounts of ACTH to monkeys in late pregnancy. Three of seven aborted, the other four gave birth to healthy offspring which were found to have very atrophic adrenal cortices, suggesting that exogenous ACTH does not enter the fetal circulation. Another study by Knobil & Briggs (197) shows however that in the rat endogenous maternal ACTH does cross the placental barrier.

The *in vivo* inhibition of (uterine and placental) histaminase in mice has a generally deleterious effect on pregnancy (198). One well-studied antihistamine drug, Pyranisamine maleate (199), has been shown not to interfere with ovulation, conception, or implantation in the mouse despite earlier claims that antihistamine drugs inhibit deciduomata formation. Folic acid antagonists do not prevent implantation but lead to fetal stunting, or death, or the birth of malformed young (200). In conformity with earlier findings 122 infants born to diabetic mothers weighed on the average 550 gm. more and were 1.5 cm. longer than the average figures for an equal number of infants born to nondiabetic mothers (201).

Smith & Smith (202) have come to a vigorous defense of the value of prophylactic estrogen therapy which they introduced for the management of threatened abortion. Against their report based on an impressive clinical experience is a mounting number of well-controlled studies showing no benefit from such treatment [Swyer (203)]. Peck (204) reports that the Smith regimen produced no significant change in mean erythrocyte count, hemoglobin, or hematocrit values, but some patients showed alterations in serum iron, iron-binding, and erythrocyte protoporphyrin, and some developed anemia. Also, contrary to White's similar use of estrogen, there is the report of Peel's study (205) under the direction of the British Medical Research Council in which stilbestrol was administered to a controlled series of preg-

nant diabetic mothers without reduction of fetal loss or of incidence of complications.

The functional activity of the adrenal cortex is known to increase during pregnancy. To this the further information may now be added that the plasma level of 17-hydroxycorticosteroids [Bayliss *et al.* (206)] and of 17,21-dihydroxy-20-ketosteroids [Robinson *et al.* (207)] is elevated during pregnancy, and that terminally the plasma neutral 17-ketosteroids are reduced [Gardner *et al.* (208)]. In general agreement also are the observations that tubular reabsorptive capacity for chlorides is increased in pregnancy (209), that the glucose tolerance is decreased and the resistance to insulin increased in the last trimester of pregnancy (210), and that uropepsinogen generally is elevated (211). The metabolism of pregnancy and especially that pertaining to the fetus has received much attention (2, 3, 4). Huggett (212) has enlarged upon the previous basic discovery that in ungulates, both glucose and, in larger measure, fructose are present in the fetal blood stream. He has also adduced new evidence that the rapid passage of sugars across the placenta may involve an active transport mechanism (213) and has contrasted the placental glycogen and phosphatase relations in rodents and ruminants (212). His group (214) has also explored more deeply the apparent ability of the fetus to utilize glucose anerobically. Burwell (215), in a review of the work of his laboratory, enumerates the important physiological changes found to occur in the pregnant woman: oxygen consumption increases to 20 per cent above the nonpregnant level; heart rate is up 10 beats per min. by the thirtieth week then slowly declines; there is a slight but significant fall in diastolic pressure; cardiac output may rise as much as 40 to 50 per cent by the thirtieth week, after which it declines to term; there is a rise in blood volume to the thirty-second week with subsequent decline and an increase in total body water which persists to term. There is also during pregnancy a progressive decline in serum protein values [Brown (216)] which is almost entirely attributable to a diminution of the albumin fraction. A reasonably reproducible eclampsia-like syndrome has been obtained in rats by administering renin to animals with existing sodium-DCA hypertension [Page & Glendening (217)]. Sodium itself, however, plays the final determinant role in this experimental syndrome. Such animals show proteinuria, oliguria, generalized edema, and convulsions. The primary lesion appears to be a depolymerization of the mucopolysaccharide ground substance throughout the body. The serum antidiuretic activity is reported to be heightened in the ninth month of human pregnancy [Croxatto *et al.* (218)], but Hofmann *et al.* (219) were able to show that pregnancy does not affect the rate at which intact or hypophysectomized rats dispose of a water load.

*Amniotic fluid.*—By measuring the rate of transfer of radioactive hydrogen, sodium, and potassium from the amniotic sac and the concentration of these substances in amniotic fluid in women at term, Hutchinson *et al.* (220) were able to substantiate a previously advanced assumption that amniotic fluid is not a transudate or dialysate of maternal plasma. Each element exchanged at its own rate and remained in dynamic equilibrium with the

maternal system. New detailed analyses of the composition of amniotic fluid, early chorionic fluid, and the fluid of hydatiform moles has been reported (221, 222, 223); McKay *et al.* (223) describe an "osmotic gradient" in which the osmotic activity of chorionic fluid exceeds that of amniotic fluid, and this in turn is exceeded by maternal serum. Water is therefore concentrated in amniotic and chorionic fluid against the higher osmotic pressure of the environmental fluid.

*Pregnancy tests.*—On the basis of accuracy, economy, simplicity of technique and speed of performance, Benitez *et al.* (224) state a preference for the serum test using *Xenopus laevis* (*Hogben*), to the urine test using male *Rana pipiens*. Farris (225) has introduced a new means of diagnosing pregnancy based on a change in the color of the cervix. The vaginal smear technique remains unsuited for the establishment of a diagnosis of pregnancy in women (226) but it has been used successfully in mares after the twentieth day of gestation (227). That a diagnosis of pregnancy could not be made in cows (228) by testing dialysed or filtered fecal solutions on male frogs will hardly arouse doubt. A note by Tabarelli-Neto *et al.* (229) quite appropriately calls attention to the hazard in pregnancy testing introduced by their finding that the injection of oxalic acid and oxalates into toads yields a positive spermiation test; ergo, plasma treated with oxalate as an anticoagulant is unsuitable for use in this test.

*Placenta.*—Fresh full-term placentae homogenized and separated by centrifugation into cell fractions show the chorionic gonadotrophin to be distributed as follows: slightly more than one-half in the microsome and soluble cytoplasm fraction, one-fourth in the nuclear fraction, and less than one-fourth in the mitochondrial fraction [Siebert & Stark (230)]. Assali & Hamermesz (231) bring forth new evidence that the placenta secretes corticotrophin. Evidence for the presence of several steroids more polar than progesterone in extracts of placentae, placental fluids, and pooled pregnancy plasma has been cited (232); one of these had high progestational activity by the Hooker-Forbes test. Gordon & Villee (233) find that the effect of cortisone *in vitro* on human placental tissue is to decrease glucose utilization and hasten the disappearance of glycogen. Placental weight in guinea pigs is inversely related to the number of fetuses (234).

Studies relating to placental permeability show: (*a*) rapid mother-to-fetus and fetus-to-mother transfer of glucose in the guinea pig and a slower transfer of fructose (235); (*b*) mother-to-fetus and fetus-to-mother transfer of $I^{131}$ in rats and rabbits (236); (*c*) a ready, perhaps preferential mother-to-fetus transfer of $Ca^{45}$ in swine, but no transfer of $Mo^{99}$ (237); (*d*) mother-to-fetus diffusion of carbon monoxide, slowly reaching equilibrium (238); and (*e*) inhibition of the transfer of iron from mother to fetus in the rat in excess of the total needs of the fetus (239).

## REPRODUCTION IN THE MALE

*Testes.*—Immature rat testes have been frozen to −79°C., thawed, and shown to be capable of forming scrotal homografts with ability to generate spermatozoa (240). The application of temperatures of −6° and −8°C.

directly to the testis for 1 hr. led, however, to irreparable disruption of spermatogenesis, and temperature of −10°C. applied to the scrotum caused severe but reparable tubular damage [Macdonald & Harrison (241)]. The damage was believed to result from cold-induced ischemia. By the same token, interruption of the arterial supply to the head of the epididymis in rats led to occlusion of the sperm duct and severe testicular atrophy, accompanied, as shown for the first time since the disproof of Steinach's masculinizing operation (vasoligation), by a significant absolute decrease in total volume of interstitial cells [Harrison & MacMillan (242)]. Persisting peritubular nodules of undetermined nature were found in monkey testes after prolonged interstitial cell stimulation (243). The testis atrophy induced in young rats by testosterone results in an absolute increase in total cholesterol in young rats, whereas large doses promote a decrease in total cholesterol and the expected acceleration of spermatogenic activity (244). Rat testicular hyaluronidase was severely reduced by hypophysectomy, cryptorchidism, or estrogen treatment, but not by testosterone propionate, which had, moreover, a protective action [Steinberger & Nelson (245)]. The rate of decline in hyaluronidase activity was found to bear a close sequential relation to the rate of degeneration of the seminiferous tubules. Maqsood & Parsons (246) found no difference in weight of testes or fructose content of seminal vesicles between groups of male rabbits kept in continuous light or continuous darkness for periods up to 20 weeks. Suzuki *et al.* (247) gathered evidence showing the direct transportation of testis hormone to target organs adjacent to the testis. An expert account of sexual functions in the male toad has been prepared by Houssay (248), and an instructive diversion on the origin of the term ridgling by Forbes (149).

Further work in the antifertility field has uncovered the capacity of triethylene melamine to maintain complete sterility in male rats (250) and has shown that the arrest of spermatogenesis induced by nitrofurans is coupled with an inability of the more mature germinal elements to oxidize pyruvate *in vitro* (251). The feeding of 80 to 140 p.p.m. molybdenum induced a high degree of sterility in male rats but not in females (252). The progressive involution of the germinal epithelium seen in Vitamin-E-deficient male rats and rabbits has now been described in similarly deficient male guinea pigs (253). Again, the order in which the germ cells degenerated was the inverse of their origin.

Socially raised male guinea pigs have a higher level of sexual behavior than that attained by males reared in isolation. Ten weeks after their castration, androgen treatment restored sexual behavior only to the precastration level peculiar to each group. The background of experience determines, therefore, the extent of the behaviorial response to androgen [Valenstein & Young (254)]. Contact with males fosters the early development of sexual behavior patterns in young male guinea pigs nearly as effectively as contact with females (255).

The cervix (256) and uterotubal junction are re-emphasized as barriers to the passage of sperm in the female genital tract (257, 278). Exceedingly few sperm were found to reach the fertilization site in rabbits and sows re-

spectively. The fertilizing capacity of rabbit sperm is brought to maximum by incubation in the uterine cavity for 6 to 16 hr. and is lost by 30 hr. (259). The cloacal protuberance in birds is thought to serve scrotal-like thermoregulating functions for the sperm storing ducts (260). The testes of men with idiopathic eunuchoidism and with intrasellar tumor- or estrogen-induced hypopituitarism are described (261); as usual the severity of the testicular atrophy was related more to the degree than to the duration of the hypopituitarism. Louw (262) has written disparagingly of the practice of using hormones in ascertaining the cause of *non decensus*. New data further substantiate the high incidence of tumors in cryptorchid testes [Thomas & Bischoff (263)] and emphasize the poor prognosis as to development of reproductive function [Alnor & Hartig (264)] though signs of degeneration were not evident in the testes of unilateral cryptorchid boys prior to puberty [Andersen *et al.* (265)].

The cryotolerance of sperm for veterinary purposes has been further elaborated (266, 267). Human sperm have not survived freezing and thawing (268). As diluents for spermatozoa in the artificial insemination of farm animals the following have been used: whole egg citrate (269), glycine-egg yolk (270), cows milk and honey (271), and physiological saline at pH 6.0 (272).

Mann (273) has provided a comprehensive but concise review of the biochemistry of semen with particular attention to the enzymes and proteins of seminal plasma and the origin and metabolism of seminal fructose. The constituents of bull seminal plasma have been analyzed in considerable detail by Rothchild & Barnes (274). Fructose determinations of semen in cases of infertility continue to be unpromising as a diagnostic procedure (275). It is claimed that the treatment of bulls with Vitamin E improves semen yield and its fructose content (276).

*Androgens.*—The protein-anabolic action of testosterone propionate was found to be evident in hypothyroid rats [Kochakian & Dolphin (277); Rupp & Paschkis (278)] and was matched in castrated animals by 19-nor-testosterone cyclopentylpropionate [Stafford *et al.* (279)]; a single injection of the latter produced significant N retention lasting several days. The weight-loss regularly occurring in kidney and heart after castration as well as the proportionate decrease in adenosinetriphosphatase and transaminase were restored by testosterone (280); liver arginase, adenosinetriphosphatase, transaminase and alkaline phosphatase were not, however, affected by the hormone. Folic acid was reported to enhance the stimulating action of testosterone on male sexual accessories (281). Burstein *et al.* (282) report that testosterone-3-$C^{14}$ in male guinea pigs yields 3-$\alpha$ hydroxyetiocholan-17-one and epiandrosterone as metabolites. Of the radioactivity of the injected dose, 83 per cent was recovered in the urine. Harrison *et al.* (283) found the amount of biologic androgen in the urine of patients to be increased by ACTH and reduced by bilateral adrenalectomy even in castrated subjects. It was reported that the comb response to testosterone was somewhat lessened by keeping the test animals in window pane-filtered sunlight and that a similar

depressing effect was seen in capons exercised on a treadmill for 15 min. periods twice daily for 10 days [Wong (284, 285)].

### Male Reproductive Tract

Testosterone propionate caused a greater increase in intracellular than in extracellular water of the seminal vesicles and prostate gland [Rudolph & Starnes (286)]; "sodium space" water reached a peak at 24 hr., suggesting an imbibition effect, then fell through the ninety-sixth hour as growth of the structures continued. The $Q_{O_2}$ of rat seminal vesicles and prostates was found to be diminished by castration and quickly restored by testosterone; this action of testosterone was not inhibited by hydrocortisone (287). It may be meaningful that ketose reductase has been found only in those rat accessory sexual glands which accumulate fructose [Williams-Ashman & Banks (288)]. The appearance of fructose in such glands coincides with the initiation of secretion and in conformity with previous opinion is almost certainly under the control of testicular androgen. Moreover, any androgen which supports the growth of these accessory glands also maintains fructose production [Levey & Szego (289)]. According to earlier work by the Manns, free fructose is produced in the seminal vesicles as follows: glycogen→1-phosphoglucose →6-phosphoglucose→6-phosphofructose →fructose. The distribution of the necessary phosphorylating enzymes in the accessory glands is in accord with these requirements [Rollinson (290); Brandes & Bourne (291)]. Glycogen secretion by the epididymal epithelium has been demonstrated [Nicander (292)], and spermatozoa are known to be able to utilize glycogen as well as glucose and fructose.

The castration of rat fetuses at very early stages did not forestall the differentiation of prostatic buds, but their number was reduced, and the coagulation gland was sometimes absent. These changes could generally be prevented by exogenous androgen suggesting that the fetal gonads may be hormonally functional [Wells (293)]. The genetically female fetuses in pregnant monkeys chronically treated with androgen are rendered permanently pseudohermaphroditic [Wells & Van Wagenen (294)].

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# THYROID[1,2]

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The functions of the thyroid gland are to elaborate, store, and secrete iodinated hormones. These hormones are carried away by certain blood proteins and distributed among all the tissues. Their mode of action is not yet known, but there is no doubt, however, that the iodine contained in their molecule is involved, so that a review on the thyroid gland should include not only recent information on the biosynthesis of the hormones but also data on their utilization; that is, it should cover the general problem of iodine metabolism. With the limited space available here it is not possible to present all the progress which has been made in the last two years. This review will be an addition to the excellent article of Barker published in the 1955 *Annual Review of Physiology* (1). The subjects treated there in great detail will only be outlined here, especially hormone regulation and embryonic development and tumorigenesis in the thyroid; other points, such as the nature of the iodinated amino acids in the thyroid, their secretion, their general and hepatic metabolism, and the activities of new synthetic compounds having hormone properties will be developed at length. The reader might consult certain recent general reviews (2 to 8) containing many references. These include the physiology as well as the biochemistry and the pathology of the thyroid and of the hormone products it secretes.

Three main aspects will be covered here: (*a*) metabolism of iodine in the thyroid, (*b*) nature and transport of the circulating hormones in plasma, and (*c*) metabolism of the thyroid hormones and biological activities of the iodothyronines and related compounds.

## THE THYROIDAL IODINE

It is generally considered that the thyroid gland is made up of an association of a great number of follicles which are each functional. The follicle is a spherical mass of cells surrounding a viscous liquid, the colloid, containing thyroglobulin, an iodinated protein. The synthesis of thyroglobulin and of the hormones contained in it necessitates the previous concentration of

[1] The survey of the literature pertaining to this review was completed in June, 1955.

[2] The following abbreviations have been used in this chapter: DIT (3,5-diiodotyrosine); MIH (2- or 4-monoiodohistidine); MIT (3-monoiodotyrosine); TSH (thyroid-stimulating hormone). For the iodothyronines the numbers preceding them give the position of the iodine atoms, for example: 3-MITh (3-monoiodothyronine); 3,3′-DITh (3,3′-diiodothyronine); 3,3′,5′-TRITh (3,3′,5′-triiodothyronine); PBI (protein bound iodine).

[3] My great thanks are due to my co-worker, Mr. Volpert, who translated this paper from the original French text. His knowledge of the subject proved to be of great help.

blood iodide by a trapping mechanism which is still not quite clear: where and how does it function?

## Site and Mechanism of Thyroidal Iodide Trapping

*Site of iodide trapping.*—Our data on the site of thyroidal iodide trapping have been obtained chiefly by applying radioautographic methods (9) to radioactive thyroid slices. There are two ways of assembling the tissue slices. In one the thyroid fragments are fixed; all iodine containing compounds soluble in water and organic solvents are eliminated. In the other the freezing drying method is used; this leaves in all the radioactive iodinated products. Leblond & Gross (10) have shown that radioactive iodine was present in the colloid and in the epithelial cells 1 hr. after injection; later it was found in the colloid only, mainly in an organic form, implying that uptake first took place in the cells, and only later was there concentration in the colloid. Quantitative studies have shown that the small follicles generally localized in the center of the gland are especially active (11). Then for the entire thyroid, iodine-incorporation depends on the total cell volume of its follicle agglomerates. The formation of thyroglobulin in the shark has been followed by radioautography at various intervals after administration of $I^{131}$ (12). From the first hour on all the iodine is present in the colloid, and there is none in the epithelial cells; this implies that all the iodination reactions take place in the vesicles. Pitt-Rivers & Trotter (13) have obtained interesting results. Giving radioactive iodide to rats treated previously with propylthiouracil, they showed that the gland concentrates inorganic iodide in the colloid and perhaps in the epithelial cells. Recently Wollman & Wodinsky (14) have restudied the concentration problem using mice. At varying lengths of time after injection of $I^{131}$ the organic iodine is concentrated in the colloid only. This is so even after an extremely short time, 11 sec., and this leads one to suppose that the iodination of thyroglobulin does take place in the colloid. Actually these two latter studies are complementary. The first was done on rats whose thyroids were not normal as a result of previous thiouracil treatment; in that case the iodination reaction in the colloid would not result in organic iodine compounds but more likely in the oxidation of the goitrogenic substance from which iodide would be derived. However the work of Wollmann & Wodinsky, especially as far as the short time experiments are concerned, was done with such a high radioactivity that damage to the follicles could not be excluded since there are serious chemical and histological changes in the thyroid after administration or exposure to radioactive sources (15, 16). Lindsay *et al.* (17) have observed thyroid lesions after irradiation. First there are changes in the epithelium, with vascular necrosis and thrombosis; then there is atrophy of the follicles with perifollicular fibrosis. Hyperfunctioning thyroids also have histological changes.

*Mechanism of iodide trapping.*—Penetration of iodide into the follicle is necessarily followed by its oxidation to iodine. To learn more about the

mechanism which follows its fixation it would be interesting to relate it to concentration of other halogens by the gland; this would indicate whether it was specific or not. Baumann & Metzger (18) have studied the ability of the thyroid to retain some elements of Group VII. Radioactive isotopes of fluorine and bromine were used in these researches. $F^{18}$ is not concentrated (19). In an early work by Perlman *et al.* (20) with $Br^{82}$, repeated by Verkhovskaya (21), it has been shown that the radioactivity of the thyroid by unit weight is higher than that of blood at the end of 24 hr., less than 0.03 per cent of the given dose being recovered in the gland. Yagi *et al.* (22) confirmed the presence of a bromide concentrating mechanism, but it is effective only for very small quantities of Br (1 $\mu$g), and even then its intensity, 0.5 per cent, is much less than for iodide. Bromide retention is at a maximum 6 to 8 hr. after injection, and the bromide remains in an ionizable form. No organic brominated compounds are found.

Similar results have been obtained with astatine, $At^{211}$ (23), element 85. It has been clearly established that $At^{211}$ is accumulated, though in quantities much lower than for $KI^{131}$ (24), by the thyroid gland of rats, guinea pigs, monkeys, and man (25). In 90 min. concentration is slightly over 1 per cent; the maximum is reached in 8 hr., and there is a decrease after that. Irradiation by $At^{211}$ has effects quite similar to that by $I^{131}$ (26) or $I^{132}$ (27). $At^{211}$ seems to be concentrated mainly as the element; the presence of organic compounds has not been proven (28, 29). It can be questioned whether or not the susceptibility to oxidation is similar for astatine and for bromide; the latter is more difficult to oxidize than iodide. There is also thyroid pick-up of elements of Group VII B, that is manganese, technetium, and rhenium. The latter has been used in biological studies, thanks to the radioactive isotope $Re^{188}$ obtained by irradiating stable rhenium in a nuclear reactor. The rat thyroid concentrates $Re^{188}$ selectively but in much smaller amounts than $I^{131}$ (30) and not in organic form, since substitution reactions on proteins are excluded for elements of Group VII B. This property would tend to show that there are two processes in the thyroid follicle, a fairly inefficient one able to concentrate the various halogens, and a more powerful one able to oxidize mainly iodide.

This hypothesis is not at all contradictory to that of Vander Laan & Caplan (31) who believe the iodide pick-up by the gland to be a mechanism independent of its storage. The uptake would be partially autonomous, while the accumulation would be dependent upon TSH, which might be an explanation for the fact that blood iodide concentration is inversely proportional to its binding in an organic form. Thyroidal iodide concentration depends on many other factors such as serum iodide level (32, 33, 34) and iodine content of diet (35, 36, 37). The $I^{131}$ uptake was increased in patients with impaired liver function (38), decreased after hypoxic hypothermia (39), during hibernation (40) and under various conditions of stress (41).

*Organic binding of iodine.*—In parallel with *in vivo* research, work has been undertaken on the incorporation of iodine *in vitro*. About 10 years ago

Chaikoff and co-workers (42) showed that surviving thyroid slices were able to attach radioactive iodide in an organic form. Roche *et al.* (43) extended these results to normal human thyroid slices from a patient having died in an accident. The influence of various factors on the uptake of iodine by fresh sheep thyroid slices has been studied. Aerobic conditions were necessary for iodide concentration, and many compounds which react with SH groups were found to diminish the iodide gradient (44). Organic iodine binding was inhibited by 1-methyl-2-mercaptoimidazole (45, 46). Recently formation of protein iodine (47) has been obtained with homogenates and mitochondria after incubation with $I^{131}$. For this incorporation of iodine, copper and tyrosine have to be added according to Weiss (47). Fawcett & Kirkwood (48, 49) consider copper to be only a mechanical oxidizing agent to transform iodide to iodine. They feel that fixation on proteins is catalyzed by an enzyme "tyrosine iodinase" which transfers the iodine to the tyrosine. The presence of 3-monoidotyrosine (MIT) has been shown by paper chromatography; only the added tyrosine, as a free molecule then, is acted upon by the iodine; there is no effect on the tyrosine groups within the proteins of the thyroid extract; without added copper or tyrosine, iodination is negligible. Wyngaarden & Stanbury (50) have suggested that the oxidation of iodide is chiefly enzymatic, enhanced by copper, and dependent upon peroxide as the active oxidant. Recently Taurog, Potter & Chaikoff (51) have obtained quite different results. They find that copper and tyrosine are not necessary for organic iodine formation in the presence of thyroid homogenates or of various separate cell fractions. Cell-free preparations are able to change important quantities of radioactive iodide to an organic form; MIT is the chief constituent. Their finding of traces of 3,5-diiodotyrosine (DIT) but no thyroxine is in accordance with the experiments of Fawcett & Kirkwood, but, contrary to their claims, the MIT is a part of the protein molecule and has to be liberated by hydrolysis in order to be identified by chromatography. On the chromatograms there is also an unknown iodine containing substance. This work questions the existence of a "tyrosine iodinase," especially since studies on oxidative enzymes lead to the same results. Milk xanthine oxidase in the presence of iodide, casein, and xanthine under aerobic conditions (52, 53) results in iodination of the protein, the main part of the bound iodine being MIT and a smaller part DIT. The absence of thyroxine and 3,5,3'-TRITh has been mentioned. The iodotyrosines are firmly attached to the protein molecule and are liberated from it by proteolytic enzymes. Alkaline hydrolysis results in the formation of a substance having the chromatographic properties of iodothyronines (52, 54).

Enzymatic proteolysis prevents the formation of artifacts and the deiodination of organic iodinated compounds. Since 1950 Roche *et al.* (55) have been using enzymatic proteolysis and they agree absolutely with Taurog, Potter & Chaikoff (51) when they write: "it is becoming increasingly clear that strongly alkaline hydrolysis may introduce serious error into the analysis of mixtures of $I^{131}$ compounds. Much of the earlier work based on this procedure should be reexamined." Braasch *et al.* (56) also recommend trypsin

hydrolysis. Incorporation of iodine by thyroid slices after enzymatic dehalogenation of DIT (57) results mainly in the formation of MIT and DIT, but there is no thyroxine. The presence of thyroxine often mentioned after incubation of $I^{131}$ with tissue slices is probably attributable to the formation of artifacts during alkaline hydrolysis (54). It is likely that two separate iodine binding mechanisms have to be considered, the first leading to the rapid synthesis of iodotyrosines and the second, much slower, to the formation of the hormones.

*Specific characteristics of thyroglobulin.*—Salter and co-workers (58) proposed the hypothesis that the protein of colloid was a substrate able to bind free iodine after the action of an enzymatic oxidation mechanism. Derrien *et al.* (59) prepared pure thyroglobulins, giving on analysis different values for iodine, which led them to support Salter's idea. Recently Nadler & Leblond (60) came to the same conclusion by histochemical considerations. Iodine fixation would take place on a globulin which might be in the colloid droplets found at the apices of the epithelial cells. The protein present in these droplets might be secreted into the colloid as a noniodinated protein substrate.

Research on the structure of thyroglobulin has often had as a goal the connection of the site of formation of the hormones or their secretion to special characteristics of the protein. It has been shown that peptide bonds of arginine are unusually stable in thyroglobulin. Tyrosine and iodotyrosines are quite easily freed by crystalline pepsin, which is not the case for most other proteins (61). The liberation of iodotyrosines during thyroid proteolysis might be related to this fact. The nature of the N terminal amino acids has been established: leucine, valine, glycine, serine, threonine, aspartic and glutamic acids, alanine and tyrosine and also DIT and thyroxine (62, 63). The presence of numerous N terminal amino acids might lead one to suppose the existence of several polypeptide chains and then to the existence of a branched structure. This would be in accordance with the high molecular weight of this protein; 680,000. At least two thyroidal proteins appear on electrophoretic patterns of rat thyroid extracts. One corresponds to thyroglobulin and the other is an uniodinated protein (64). Another characteristic of thyroglobulin is its glycoprotein nature. The existence of a glycoprotein in the thyroid had been supposed after histochemical studies (65). The presence of galactose and mannose in crude thyroid products has been established (66), as well as glucosamine in fairly high concentrations in partially purified thyroglobulin (67). Hooghwinkel *et al.* (67) and Boas & Foley (68) have shown by electrophoresis that thyroglobulin is a glycoprotein and Lacombe & Michel (69) have found galactose and mannose in addition to glucosamine; fucose also has been recently detected (70). The glycoprotein nature of thyroglobulin could explain its physical properties, such as its high viscosity and slow flow rate. From the biological point of view the main interest of thyroglobulin is that it is an iodoprotein which contains several iodinated amino acids, some of them being the thyroid hormones.

*Nature of iodinated compounds of thyroglobulin.*—Enzymatic proteolysis

of thyroglobulin liberates iodinated amino acids with small quantities of their peptides. Except for thyroxine and DIT which have been known for some time, the iodinated aminoacids have been identified within the last 10 years thanks to the use of $I^{131}$ and chromatographic techniques. The iodide picked up by the thyroid rapidly reacts after enzymatic oxidation with the amino acids able to bind iodine, namely tyrosine and histidine, to give MIT (71), DIT, and MIH (72), chemical models of which are relatively simple (73). The halogenation reaction proceeds in two steps; first the monoiodinated derivative is produced and is then transformed into the diiodo compound with excess halogen. Since the benzene ring of tyrosine is more reactive towards iodine than the imidazole ring of histidine, the former reacts preferentially in a mixture or when they are both within the same protein molecule. This explains the low MIH content of thyroglobulin. Kinetic exchange and concentrating and organic binding of iodide were examined by Wollman (75) and by Berson & Yalow (76).

In fact the iodotyrosines are of interest only as precursors or the iodothyronines since they have no biological activity. In addition to thyroxine, known and synthetized a long time ago, 3,5,3′-TRITh has been shown to be present in thyroglobulin hydrolysates (77 to 80). Its discovery raised the question of the existence of other iodothyronines, in view of the presence of small quantities of unknown iodine-containing substances in the thyroid according to the studies of Gross & Leblond (81). The difficulties encountered in the characterization of 3,5,3′-TRITh had shown that any possible step forward required obtaining synthetic substances as standards. Roche, Michel & Wolf (82) achieved the synthesis of several iodothyronines: 3-MITh, 3,3′-DITh, 3′-MITh and 3′,5′-DITh; the synthesis of this latter substance had been described earlier using another method. By working out new chromatographic solvents able to separate the various iodothyronines it has been possible to establish the presence of 3,3′,5′-TRITh and 3,3′-DIT in rat thyroid. With solvents used previously 3,3′,5′-TRITh overlapped the thyroxine spot on chromatograms. 3,3′-DITh is separated from thyroxine by a solvent composed of methanol buffered with ammonium acetate. These two substances have been shown to be present in rat thyroid 6 to 24 hr. after injection of $I^{131}$ (83, 84). The existence of these new iodothyronines and their early appearance open a discussion on the various theories of thyroid hormogenesis.

## Mechanism of Hormogenesis

According to the hypothesis proposed by Harington (85) quite some time ago it is generally accepted that thyroxine results from the condensation of two molecules of DIT with elimination of an alanine residue. Chemical models have been used to confirm this. Since iodine is a catalyst for the condensation reaction, it may be unnecessary to postulate an enzyme, but the reaction needs an energy supply. It has been shown that when labelled thyroid extract is incubated in an isotonic liquid containing diphospho-

pyridine nucleotide, the yield of thyroxine is increased (86). The mechanisms involved in the formation of the other iodothyronines are not yet quite clear. A discussion has been started on the biosynthesis of 3,5,3′-TRITh (3, 5), but it is very likely that similar processes are true for its other isomer as well as for 3,3′-DITh, and the subject would then be more general. Several ways are open to explain their biosynthesis.

The first hypothesis was to suppose that these products were formed by iodination of thyronine, but it seems now that this compound is absent, anyway the substances do not result from its chemical iodination. The phenolic group makes its benzene ring more reactive (87) and orients iodine substitution to the positions 3′ and 5′. 3′-MITh can be prepared directly by iodination, while 3-MITh has been obtained only by partial catalytic dehalogenation of 3,5-DITh. 3,3′-DITh is formed by monosubstitution of 3-MITh, and 3,3′,5′-TRITh by iodination of 3,3′-DITh; thus the iodination mechanism of thyronine cannot be considered as a possibility for formation of iodothyronines. A second hypothesis is that 3,5,3′-TRITh originates from partial halogenation of 3,5-DITh, but it has not been definitely shown that 3,5-DITh exists in thyroglobulin. The radioactive spot on two dimensional chromatograms, earlier thought to be 3,5-DITh (74), might really be 3,3′-DITh. Anyway if the recently discovered iodothyronines are formed by a similar process it would have to be supposed that they are the result of the partial iodination of 3-MITh, the existence of which is still more problematical.

The two last hypotheses explaining thyroid hormone biogenesis rest on more solid foundations. Pitt-Rivers & Gross supposed that 3,5,3′-TRITh was the result of the partial deiodination of thyroxine (88). Yet no enzyme deiodinating it has been found in the thyroid up to now. But even if such an enzyme system exists it is unlikely that it would stop its action at the 3,5,3′-TRITh stage; the iodine atoms in 3′ and 5′ are equally unstable, and in a second step the deiodination of 3,5,3′-TRITh would proceed to give 3,5-DITh, which is not definitely a constituent of thyroglobulin. And how would the other proven iodothyronines be formed? To explain their biogenesis the enzyme system would require an unusual specificity; this action would not be related to the stability of the iodine atoms in the thyroglobulin molecule.

A fourth and final possibility would be that of extending to the other iodothyronines the hypothesis suggested by Harington & Barger (85) for the formation of thyroxine. Condensation of two molecules of MIT would lead to 3,3′-DITh with the loss of the alanine residue of one of the two MIT molecules, or to 3,3′,5′-TRITh if one molecule of MIT and one molecule of DIT reacted, and the alanine residue of DIT were eliminated. The *in vitro* condensation of chemical models is certainly more difficult if the molecules are unlike, which would explain why by incubating a mixture of N-acetyl MIT and N-acetyl DIT, Pitt-Rivers & Gross (88) did not obtain the synthesis of N-acetyl 3,5,3′-TRITh, while N-acetyl thyroxine can be produced under the same conditions with two molecules of N-acetyl DIT. It is

possible, however, that the iodine formed during incubation might be fixed on the MIT molecule as soon as it appears and would therefore be unable to catalyze the condensation. The coupling of two unlike molecules is theoretically possible, but it is hard to suppose that 3,5′,3′-TRITh alone would be formed in this way; its isomer 3,3′,5′-TRITh should be formed in the same way (3). Characterization of this compound in the thyroid then adds another argument to this theory. It should be pointed out that this concept of iodothyronine formation gives an equal importance to MIT and to DIT, since MIT would be the precursor of the three partially halogenated iodothyronines. Added support to the hypothesis of 3,5,3′-TRITh formation by condensation is given by studying the kinetics of formation of the various iodothyronines (83). They all appear in the thyroid simultaneously. It seems though that 3,3′-DITh is formed before the others, while this would be the opposite if it originated from thyroxine (84).

## Hormone Secretion

Thyroglobulin as such has no hormone properties. These appear only after liberation of the iodothyronines. The iodoprotein is a biological reserve of the hormones (89). Thyroid proteolysis leading to diffusible products of low molecular weight takes place in the colloid vesicles and is carried out by proteinase systems of a catheptase nature characterized for the first time by de Robertis in 1941 (90) and later confirmed by various authors. Their activity has been tested in different substrates, and it was found that TSH activates catheptases *in vivo* (91) as well as *in vitro* (92). The hydrolysis of thyroglobulin has been studied lately using as a substrate an iodoprotein biologically labelled with $I^{131}$. These studies were started with crude enzyme preparations (93, 94), but purified thyroid proteases have been used recently (95). Hog thyroid protease has been tried on various synthetic iodopeptides (96), and there are indications that it behaves similarly to pepsin since it hydrolyzes the peptide linkage between two aromatic amino acids and its pH optimum is close to 3. From thyroglobulin it liberates iodotyrosines, traces of MIH, thyroxine, 3,5,3′-TRITh, peptides, and an unknown compound with an $R_F$ corresponding to that of 3,3′,5′-TRITh in the chromatographic solvent used [P. Stanley (Personal communication)]. Thyroxine has also been identified by proteolysis of rat thyroglobulin (97). Even though 3,5,3′-TRITh could not be found it can be supposed that it also is liberated by the proteases since it has been distinguished among the free amino acids of rat thyroid originating from thyroid proteolysis. Recently 3,3′-DITh and 3,3′5′-TRITh (98) have been identified in the butanol extracts of rat thyroids, but it is definite that the iodothyronines are not freed specifically during proteolysis.

There is a kind of physiological selection operating because of a deiodinating enzyme system. Thyroid slices or homogenates are able to dehalogenate DIT, removing iodide and forming MIT which in turn is deiodinated and transformed to tyrosine. This mechanism depends on an enzyme since

it is stopped by heating, varies with the pH, and with various chemical effectors; for instance, it is inhibited by thyroxine and activated by TSH (99). It has some specificity since it acts on the iodotyrosines, probably on MIH, but not on thyroxine, 3,5,3′-TRITh or 3,3′-DITh. Tong, Taurog & Chaikoff (100) confirmed the existence of a thyroid deiodinase. They showed it to be different from that in various other organs, like liver, since neither pyruvate nor $\alpha$-ketoglutarate stimulate DIT deiodination in the presence of thyroid homogenates or slices. Thyroid tissue does not seem to have any transamination mechanism for DIT. This observation is important as it confirms that dehalogenation leading to tyrosine is a result of the action of a specific enzyme, which would not be true if 3,5-diiodo-4-hydroxyphenylpyruvic acid had been found (101); this compound is quite unstable and loses its iodine atoms spontaneously. The iodine originating from dehalogenation of the iodotyrosines is incorporated into thyroglobulin. This led Roche and his colleagues to postulated that practically all the iodine entering the thyroid leaves it as hormone iodine but after one or several cycles of integration into thyroglobulin. Recently Taurog, Wheat & Chaikoff (102) brought forth a decisive argument in favor of this hypothesis. Having administered $I^{131}$ to horses and sheep, they analysed the plasma of the thyroid vein by chromatography and found mainly thyroxine and 3,5,3′-TRITh. There was much more of the former, and practically no iodotyrosines even though thyroglobulin is vastly richer in these than in iodothyronines. There is a mechanism selecting among the various iodoamino acids from the proteolysis of thyroid proteins; all the iodothyronines go into the circulation directly while the iodotyrosines are dehalogenated.

It can be concluded that the thyroid, in addition to being able to concentrate blood iodide, also has the ability to reclaim iodine resulting from the dehalogenation of the iodotyrosines. These act as hormone precursors and do not leave the gland. In this way the thyroid metabolizes iodine with the maximum efficiency.

## CIRCULATING THYROID HORMONES

The circulating thyroid hormones have recently been covered by a general paper (103). Only studies published since the last article will be mentioned here; however a few older concepts will be taken up if they fit in with our recent knowledge. First the nature of blood iodine and its transport will be considered, then the origin of the circulating hormones will be discussed.

*Nature of blood iodine.*—When we study the data on blood iodine we have to distinguish between plasma and red blood cell iodine. The red blood cell membrane is not permeable to thyroxine (104) or 3,5,3′-TRITh (105), and furthermore, in red blood cells from a patient who received $I^{131}$, there are no organic iodinated substances extractible by butanol after rinsing. Then red blood cell iodine can only be in the form of iodide, the fixation of which on protein has been postulated (106) but not proven with certainty.

The cell membrane is permeable to iodide, but the red cells do not contain any protein bound iodine in patients with thyrotoxicosis (107). We must suppose all of the organic iodinated compounds in blood to be present mainly in plasma.

White *et al.* (108) and Courrier *et al.* (109) have worked out a clinical thyroid function test based on the permeability to iodide of the red cell wall and its impermeability to organic iodine-containing compounds. Courrier *et al.* (110) studied the test in the rat and showed that 24 hr. after injection of $I^{131}$ the ratio of the radioactivities, blood cells/plasma, for the same weight, is directly related to the functional state of the thyroid gland. This ratio, 0.14 in a normal animal, goes up to 0.60 after hypophysectomy.

The nature of plasma iodine is not entirely clear. Besides iodide, 10 to 20 per cent of the total iodine, there is no doubt any more about the presence of thyroxine, 70 per cent of the total. 3,5,3′-TRITh has been identified (77) but does not seem to be a permanent constituent, which might explain why some authors have not found it (111, 112). The low specific activity often makes studies difficult, and a previous purification is needed to obtain good chromatographic records (113), even though according to Acland (114) an exchange reaction might be possible. Cettini *et al.* (115) have consistently found 3,5,3′-TRITh in blood of hyperthyroid patients and mentioned in some the presence of MIT. Robbins *et al.* (116) have established the presence of thyroglobulin giving MIT and DIT by hydrolysis in the plasma of patients having thyroid cancer and receiving large doses of $I^{131}$. The presence of thyroid iodoprotein has been confirmed by salting out and by the ultracentrifuge sedimentation rate of whole serum proteins (117, 118).

The existence of unknown substances has also been mentioned. Recently Roche *et al.* (119) found 3,3′-DITh in the plasma of rats treated with $I^{131}$ 15 to 24 hr. previously. The presence of this compound has been established by radiochromatography in two successive solvents. Practically overlapping the thyroxine spot in *n*-butanol-dioxane-ammonia, it is separated from this substance by methanol buffered with ammonium acetate. There are several spots on radioautograms of bidimensional chromatograms; they correspond to thyroxine, 3,3′-DITh, iodide, and an unknown compound. This substance has an $R_F$ very close to that of the iodotyrosines in *n*-butanol-dioxane-ammonia and could be considered as being DIT or MIT if its $R_F$ in methanol-ammonium acetate did not exclude this supposition. The 3,3′-DITh level in plasma might go as high as one-fourth of the thyroxine present, just as in thyroid. It appears quite early, since it is found from the fifteenth hour after $I^{131}$ injection, and its blood level seems to go down later. 3,3′,5′-TRITh should be present also, but probably in such small quantities as to make its identification very difficult.

*Plasma protein binding of iodinated compounds.*—The organic substances in plasma are bound in an unknown manner to certain proteins. Nevertheless PBI determination (120) remains, with the estimation of $I^{131}$ thyroid pickup (121), as one of the tests most widely used for the diagnosis of thyroid dis-

eases or for comparing thyroid function in various animals (122). It rises after major surgery, and after treatment with ACTH (123) or epinephrine (124). During the last few years experiments have brought attention to the question of transport. By paper electrophoresis Gordon *et al.* (125) proved thyroxine to be associated with the $\alpha_1$-globulins. Various authors have confirmed this role of the $\alpha$-globulins in plasma. A substance migrating in paper electrophoresis between the $\alpha_1$ and $\alpha_2$ proteins takes part in the reaction. Schmidt (126) and Robbins *et al.* (127) established this material to be a glycoprotein. Endogenous thyroxine of patients receiving radioactive iodine is at first bound in part to the albumin but goes down in time and stabilizes itself on the $\alpha_2$-globulins. According to Larson *et al.* (128) the fraction of iodine bound to the albumin is not extractable by butanol, which would exclude its identification with iodothyronines. The sedimentation rate of the thyroxine binding complex in whole serum has been established (129). *In vitro* 3,5,3′-TRITh seems to be attached to the same proteins as thyroxine but might be displaced by it (130); with an excess of 3,5,3′-TRITh (131), the contrary may also be true. With thyroxine the force of binding to the $\alpha$-globulins is greater than with 3,5,3′-TRITh; 3,5-DITh, DIT, and iodide had no significant binding effect. The amino, carboxyl, and phenolic groups are essential for the binding of thyroxine (132). This fact has a great biological importance; the diffusion of thyroxine into the tissues is delayed since in a sense it is slowed down by its binding with the plasma proteins. Its passing into the cells must constantly be the result of displacement of equilibrium in favor of the intracellular proteins. The more labile binding of 3,5,3′-TRITh might mean it will penetrate more easily.

*Origin of circulating hormones.*—A short time after the characterization of 3,5,3′-TRITh in blood the question came up as to its origin. Does it originate in the tissue and is it a partial deiodination product of thyroxine, or is it actually secreted by the thyroid? The former possibility comes from an experiment carried out a few years ago by Gross & Leblond (133). They produced radioactive thyroxine by biological synthesis, that is, from plasma of animals given $I^{131}$. After injecting it into thyroidectomized mice they found several radioactive substances in the blood, among which was 3,5,3′-TRITh. There was no proof, however, that the thyroxine given was absolutely pure, since it was obtained by elution from a chromatographic spot which may have contained 3,5,3′-TRITh, and even 3,3′,5′-TRITh and 3,3′-DITh as contaminants. Others, using radioactive thyroxine prepared by chemical means, have either confirmed (134) or failed to confirm (135) these results. With Roche & Tata we (136) have studied this question under various experimental conditions. After injecting small doses of thyroxine labelled on either benzene ring, an unknown compound named S, appears in plasma. This compound S is at a maximum 12 hr. after injection. It appears in plasma regularly, whether or not the bile duct has been ligated. No spot corresponding to 3,5,3′-TRITh could be found in any of our experiments, making its formation at the expense of thyroxine unlikely. Additional work (137) was

done with really physiological doses of thyroxine; 0.2 μg. were given to either normal or thyroidectomized rats weighing 100 gm. With the latter, in addition to thyroxine, only iodide could be identified. If the reaction had stopped at the 3,5,3′-TRITh stage a spot of it should have been detected. In plasma 3,5,3′-TRITh is sometimes absent, and when it is present the quantities are always very low and do not increase if labelled thyroxine is given concurrently with $I^{131}$. It is, then unlikely that 3,5,3′-TRITh would diffuse from the tissue into the plasma. Actually, if we refer to studies on TRITh metabolism, which will be reported later, there is nothing unusual in TRITh going through the plasma again, even if it originated in the peripheral deiodination of thyroxine. The organs extract it very rapidly from the plasma, and its presence there would be so fleeting as to be extremely difficult to detect, so that for Roche *et al.* there is no doubt about its origin from the thyroid (137).

There is a different conclusion to be made from studies on myxoedematous patients subjected to pretreatment with nonradioactive thyroxine, which has the effect of completely blocking thyroid activity, and who are then given radioactive thyroxine in physiological amounts (138). Chromatographic analysis of their plasma reveals a radioactive spot of TRITh. There would be no doubt about the peripheral origin of 3,5,3′-TRITh if there were absolutely none in the blood of myxoedematous patients receiving radioactive iodine, that is if there were no possibility whatsoever of secretion by the inactivated thyroid gland.

Impartial examination of work reported here shows that it is difficult to choose with certainty between the two hypotheses on the origin of plasma 3,5,3′-TRITh, namely, that it is produced by the thyroid, or that it results from tissue metabolism of thyroxine. However, one new fact, the existence of 3,3′-DITh in plasma, reinforces the first hypothesis. 3,3′-DITh is a constituent of thyroglobulin and its biogenesis has already been discussed. The possibility was excluded that it was formed from thyroxine or 3,5,3′-TRITh by partial dehalogenation in the thyroid. These arguments are still true in the matter of deiodination in the tissues. The thyroid origin of 3,3′-DITh should be considered as the only one biochemically valid. Discussion of the origin of certain iodinated compounds in blood actually fits into a much larger and more complicated problem, namely the metabolism of thyroid hormones in the tissues.

## METABOLISM AND ACTIONS OF THE THYROID HORMONES

Progress in the study of thyroid hormone metabolism in the last few years was a result in large part of advances in analytical methods (139, 140) and especially of the achievement of radioactive preparations with a sufficiently high specific activity so that the quantities given would be close to those normally secreted by the gland. The data on metabolism are really significant only if they are obtained in experiments as close as possible to physiological conditions. According to Dempsey & Astwood (141) the daily

thyroxine secretion is from 3 to 8 μg. in the rat, but it seems that 0.6 μg. are sufficient for its needs (142); the quantity of 3,5,3′-TRITh must certainly be lower.

## General Metabolism

*Excretion and metabolism of the thyroid hormones.*—The comparative degradation of the thyroid homones has been studied. In the thyroidectomized rat 3,5,3′-TRITh is more rapidly metabolized than thyroxine. Fecal and urinary excretion is faster with the former than with the latter and for the same hormone the iodine atoms on positions 3 and 5 are more stable than those on the phenolic ring (143, 144). Maclagan & Wilkinson (145) found that urinary excretion was independent of the dose with both hormones. For fecal excretion this was the case only with 3,5,3′-TRITh, since with thyroxine the excretion increased in proportion to the quantity given. With very small doses of the two iodothyronines given to normal mice, the quantity of 3,5,3′-TRITh excreted in the urine in 24 hr. is more than double that of thyroxine. The opposite has been reported to be true for fecal excretion (146). Recently, however, it was shown that renal excretion of $I^{131}$ after administering physiological doses of DL-3,3′-DITh, labelled in the 3′ position, to thyroidectomized rats is greater and more rapid than after injecting L-3,5,3′-TRITh or L-thyroxine, and its fecal elimination is low. These facts are an example of the rapid metabolic utilization of 3,3′-DITh (147).

Rawson *et al.* (148) measured the disappearance rate of the tri- and tetra-iodothyronines on athyreotic and euthyroid human subjects and found the half life to be between 2.5 and 6 days for the 3,5,3′-TRITh and practically twice as long for thyroxine. Similar half life values on man have been reported by various authors (149, 150). In the rat they are 16.6 hr. for thyroxine, and much lower for 3,5,3′-TRITh (145).

All the results obtained this way agree on one essential fact, that the elimination of 3,5,3′-TRITh is greater and more rapid than that of thyroxine (151), the differences being of a quantitative and not qualitative nature. But questions have been raised as to the distribution in the organs, the nature of the products excreted, and the nature of the tissue metabolites.

*Distribution and fate of iodine in the organs.*—Studies on the distribution in various organs after administration of thyroxine or 3,5,3′-TRITh have shown that the latter is quickly extracted from plasma and concentrated mainly in the liver, the kidneys, and the digestive tract (152). The former is accumulated in the same tissues, but it disappears much more slowly from plasma. Comparison of the distribution of the two products labelled in various positions shows that there are no important differences in radioactivity (137); there can be, however, in the nature of the compounds formed. It should be pointed out that the neurohypophysis of some animal species concentrates thyroxine when it is given in physiological doses (153). It is the same with 3,5,3′-TRITh, (146) but it is still not possible to tell whether

the radioactivity recovered in the gland results from each of the hormones injected or whether a metabolite is formed.

Details on the distribution of the radioactive thyroid hormones in the tissues can be found in a review. Gross (146) suggested in this article that thyroxine might be deiodinated on the cell surface to 3,5,3′-TRITh, which would penetrate into the cells where it would assume its metabolic role. This hypothesis can be related to the studies on the possibility of enzymatic deiodination of thyroxine and 3,5,3′-TRITh *in vitro* as well as *in vivo*.

Maclagan & Sprott (154) described formation of 3,5,3′-TRITh from labelled L-thyroxine by liver homogenates, the dehalogenation reaching 80 per cent. They ascribe it to an enzymatic process, even though the activity remained in the liver after 2 hr. boiling. Radioautograms of chromatograms obtained after incubation of radiothyroxine with liver homogenates and original thyroxine are not very different. They both contain large quantities of 3,5,3′-TRITh so that it is hard to consider these results as fully significant. Similar experiments should be repeated with samples of pure thyroxine before a conclusion can be reached as to the enzymatic nature of deiodination. It is necessary to be sure that it is not the result of chemical reactions with substances present in tissues. The dehalogenation of thyroxine in cells is evident if one considers that among the products excreted in the bile when thyroxine is perfused through the liver are trace amounts of iodide. There are very small quantities of iodide when thyroxine is incubated with liver slices. It should also be pointed out that after incubation of thyroxine with rat kidney slices Albright, Larson & Tust (155) found 3,5,3′-TRITh on radiochromatograms, but no radioautographs were produced and the counting rates given for the 3,5,3′-TRITh spot were 5 to 40 counts/min. Larson *et al.* (156) postulated that the thyroxine deiodinating system is conditioned by the level of thyroxine in the tissues.

There is significance in the chromatographic identification of 3,5,3′-TRITh in the tissues of eviscerated animals subjected to a long treatment with thiouracil derivatives, since there is no definite proof that the thyroid completely stopped secreting 3,5,3′-TRITh (157). Hogness (135) in similar experiments with intact animals was not able to show the formation of 3,5,3′-TRITh at the expense of thyroxine. When thyroxine is injected into bile fistula dogs 3,5,3′-TRITh is present (15 per cent), and when 3,5,3′-TRITh is administered thyroxine (12 per cent) is excreted in the bile (158). Thyroxine and TRITh are concentrated in the posterior pituitary of rabbits. TRITh is unchanged, but thyroxine is accompanied by traces of the triiodo compound (159). It would be interesting to know if the unidentified iodinated compound (160) present in the cerebro-spinal fluid is a dehalogenated derivative of thyroxine or not. There is no doubt about the peripheral deiodination of thyroxine because chromatographic analysis of the urine of rats having received thyroxine shows that the main substance is iodide together with 3,5,3′,5′-tetraiodothyropyruvic acid. Up to now no definite proof has been given that it stops at the 3,5,3′-TRITh stage and that its bio-

logical activity is connected with its specific dehalogenation since the deiodinating process should go further. In that case 3,5-DITh should be found in the tissues, but the existence of this substance has not yet been shown. Its absence might mean that the dehalogenation is very rapid or else that more complicated processes precede it.

## Hepatic Metabolism

The thyroid hormones are rapidly concentrated by the liver. Albert *et al.* (161), who administered 0.6 μg. of radioactive thyroxine to rats, found 30 to 43 per cent of the dose in this organ within 1 min. which shows the importance of the liver in hormone metabolism especially in relation to a hormonal enterohepatic circulation. The problem raised by the study of liver-bile metabolism of thyroid hormones is especially that of establishing the nature of the substances eliminated in the bile, and eventually to determine their excretion rate (162, 163).

*Nature of iodinated substances excreted in the bile.*—By chromatographic analysis of bile samples obtained after injection of radioactive L-thyroxine to rats it has been shown that in addition to unchanged thyroxine, iodides can be detected. Substances not found in the thyroid also occur in bile. One of these has been identified as a glycuroconjugate, since bacterial β-glycuronidase liberates thyroxine and there is no hydrolysis if the enzyme is inhibited by saccharic acid (164). Thyroxine labelled with $C^{14}$ on the carboxyl group enabled Klitgaard *et al.* (165) to localize the conjugation to the phenol group of the amino acid. Another substance existing in bile has been recently identified by Roche *et al.* (166) as probably 3,5,3′,5′-tetraiodothyropyruvic acid; by its chromatographic characteristics it corresponds to the same product found in urine. Injection of D-thyroxine into thyroidectomized rats is followed by biliary excretion of a glycuronide of it, together with other products among which there was 3,5,3′,5′-tetraiodothyropyruvic acid (167). Endogenous formation of thyroid hormones after injecting $I^{131}$ into rats results in the elimination of glycuroconjugates, which might lead one to suppose that the conjugation mechanism would have an important physiological role; it could not be a single detoxication process but rather would be related to a factor regulating plasma iodine.

Roche *et al.* (168) have studied the products of liver-bile metabolism in rats receiving radioactive 3,5,3′-TRITh. There is great similarity in chromatograms of bile samples of rats receiving either hormone. In addition to traces of unknown compounds, four spots can be detected, two of which correspond to iodide and 3,5,3′-TRITh. A third spot has been identified as a glycuroconjugate similar to that of thyroxine (166). By preparing it in small quantities by biological means it has been shown that the conjugation took place on the phenolic hydroxyl group, since it gives a positive ninhydrin and a negative Pauly reaction. Spleen β-glycuronidase regenerates 3,5,3′-TRITh, and after hydrolysis with dilute hydrochloric acid it has been possible to identify glycuronic acid after chromatographic separation by a

specific color reaction. Finally a fourth radioactive constituent separated can be considered as the α-keto acid derived from 3,5,3′-TRITh by oxidative deamination, namely 3,5,3′-triiodothyropyruvic acid. It was identified by a series of reactions; the negative ninhydrin reaction meant the absence of a free amino group, and the α-keto acid reactions and the Pauly reaction were positive (169). Important differences in the rate of biliary excretion of the two hormones were observed. With physiological doses of 3,5,3′-TRITh the bile contains a high percentage of the free hormone with only a small part as its β-glycuronide. Under the same conditions thyroxine is chiefly excreted as glycuroconjugate (136).

Recently Roche *et al.* (147) found that after injection of 3,3′-DITh into rats the substances eliminated in the bile were of the same kind as for the two other hormones. There was mainly unchanged 3,3′-DITh with small quantities of iodide and a glycuroconjugate.

The physiological significance of the liver-bile metabolism of thyroid hormones can be summarized as follows: the thyroid hormones enter the liver as free amino acids. One part is retained there, another is eliminated in the bile unchanged, and a third larger part undergoes various metabolic modifications. This means that iodide, glycuroconjugates, and iodothyropyruvic acids pass into the intestine. A part of the free amino acids and iodide, and a part or all of the keto acids are reabsorbed directly, while the glycuroconjugates are first hydrolyzed by the β-glycuronidases of the intestinal bacteria and then their amino acids are partially reabsorbed. This has been well studied by Briggs, Taurog & Chaikoff (162) and its kinetics by Albert and Keating (163). The substances in excess not reabsorbed, mainly thyroxine and 3,5,3′-TRITh, are rejected with the feces which then contain thyroxine, while most of the free amino acids and free iodide return into the general circulation. The iodides are fixed by the thyroid or excreted by the kidneys. The keto acids derived from 3,5,3′-TRITh and also probably from thyroxine seem to be concentrated and eliminated in the urine, but their formation in the kidneys is not excluded. Thus thyroid hormone metabolism is based on three essential processes: dehalogenation in the tissues, oxidation in the liver, regulation by the kidneys.

*Intracellular distribution.*—Tissue metabolism of thyroid hormones has been especially studied in the liver; for the same organ data concerning their localization in the cell particulates are available. Lipner *et al.* (170) showed that labelled thyroxine penetrated the cells both *in vivo* and *in vitro* and that it was distributed in all fractions. Tata (105) compared the rate and the percentage of radioactive thyroxine penetration with that of radioactive sulfate in surviving liver slices; the cells are practically impermeable to sulfate while thyroxine is rapidly extracted from the medium. Lee & Williams (171) showed that after an intravenous injection of labelled thyroxine there is accumulation within the liver cells in the cytoplasmic and nuclear structures. Five minutes after an injection almost 50 per cent is recovered in the mitochondria fraction, going down to 40 per cent after perfusion, which in-

dicates that the fixation is not very efficient. The injection of $I^{131}$ results in an equal distribution inside and outside the cells; the radioactivity of the cellular elements indicates that the trapping mechanism is a passive one, while it is an active one for thyroxine. Tabachnik *et al.* (172) also studied distribution of endogenous and exogenous labelled thyroxine in particulates of rat liver. These results indicate that thyroxine is attached to both particulate and nonparticulate fractions of fragmented cells. Its absence in the mitochondria and its great concentration in the supernatant fraction might explain its influence on coenzyme A synthesis, since this supernatant is the main site of biogenesis of this compound. Thyroxine increases the coenzyme A content of the liver of thyroidectomized (172) and hypophysectomized (173) rats. 3,5,3′-TRITh has a similar effect but is more active (174) just as in all other tests. Klemperer (175) studied the uptake of thyroid hormones by rat liver mitochondria in the presence of serum. Both 3,5,3′-TRITh and thyroxine are rapidly concentrated by the mitochondria. Then an equilibrium is established and the mitochondria swell. 3,5,3′-TRITh is concentrated more rapidly and more intensely than thyroxine, but the fixation is not entirely reversible. The uptake is independent of the temperature. It goes on just as well with D as with L form, and even occurs after denaturation of mitochondria. Distribution between the serum and the mitochondria seems to be a passive phenomenon, attributable simply to the union of the positive and negative valences of the thryoid hormones and the plasma proteins. Since the quantity of thyroxine required to uncouple oxidative phosphorylation is larger *in vitro* than *in vivo* it might mean, according to Klemperer, that probably neither thyroxine nor 3,5,3′-TRITh are the forms of the hormone utilized in the tissues.

### Biological Actions of Thyroid Hormones and Related Compounds

*Actions of iodothyronine derivatives.*—Thyroxine, 3,5,3′-TRITh, and 3,3′-DITh can be considered as thyroid hormones if a hormone is the substance secreted into the blood stream by an endocrine gland and which has all the biological activity of the gland. This biological activity has two aspects, first the comparative potencies of the different hormones and second the nature of the substances acting in the tissues.

In a table Barker (1) has gathered all the data up to the beginning of 1954 on the comparative activities of L-thyroxine and L-3,5,3′-TRITh. Generally the latter is 3 to 10 times as active as the former, depending on the test used. Since that review these results have been confirmed by biological (176) and by clinical investigations (177). L-3,5,3′-TRITh has been used therapeutically on patients with myxoedema; it seems to be effective more rapidly than thyroxine when given parenterally. Biological tests agree with the increase of BMR of patients. Zondek *et al.* (178) have given L-3,5,3′-TRITh orally to hypothyroid patients and studied the clinical effects. In the patients treated there was improvement of all the symptoms attributable to hypothyroidism and a rapid return to the euthyroid state. The quantities

needed are very small and the effect is quite fast; these authors point out that there is reduction of the goiter and disappearance of several nodules.

The action of 3,5,3′-TRITh on the pituitary has been investigated. Giving DL-thyroxine to rats results in the thyroid gland becoming less active; there is then much less fixation of blood iodide. Results are similar in man after L-thyroxine or L-3,5,3′-TRITh administration (179). A daily oral dose of 8.8 μg. during seven days given to normal subjects results in a 75 per cent decrease in radioactive iodine fixation, as compared with untreated subjects. There is a decrease in protein-bound iodine (PBI) in myxoedematous patients receiving 3,5,3′-TRITh. They return to the euthyroid state but without increase in PBI. Thyroxine seems to disappear and be replaced by 3,5,3′-TRITh. There is no great decrease in $I^{131}$ thyroidal pickup if a dose 15 times larger is given, but if the dose is halved there is hardly any difference with or without treatment. Entirely different results have been obtained with hyperthyroid subjects. Werner & Hamilton (180) showed that 2 mg. 3,5,3′-TRITh was ineffective and failed to suppress $I^{131}$ uptake in patients with Graves' disease. Results are similar with patients having a normal thyroid but showing exophthalmia.

Courrier *et al.* (181, 182) have found that daily injections for seven days of physiological doses of L-thyroxine or L-3,5,3′-TRITh to a normal rat greatly decreases blood iodide fixation by the thyroid. In this as well as in other aspects the latter is more active than the former. This is not the case for hypophysectomized rats. Inhibition of uptake seems to be a result of a blocking of TSH secretion and not to an antagonism between TSH and thyroid hormones.

Recentiy 3,3′-DITh has been shown to be present in the blood. Its biological activity has been studied with different tests as an antigoitrogenic substance (183) and as a substance which accelerates the metamorphosis of amphibians (184). In both cases its activity is about 80 per cent of that of thyroxine. 3,3′,5′-TRITh is much less active since it is only about 5 per cent of that, 100 times less than the other isomer.

In addition to these substances shown to be secreted by the thyroid, numerous structural analogues of thyroxine and 3,5,3′-TRITh have been studied. Two are particularly interesting. They are the related iodothyroacetic acids. Thibault & Pitt-Rivers (185) have recently shown that these increase oxygen uptake of surviving rat kidney slices. The effect is immediate; there is no latent period as for thyroxine and 3,5,3′-TRITh. The maximum effect is reached in 15 min., and it disappears after 90 min. On the basis of these *in vitro* results they propose that the iodothyroacetic acids would be the active form of the thyroid hormones. To confirm this hypothesis their presence in the tissues would have to be proven first. On the other hand the related iodothyropyruvic acids are among the liver-bile and perhaps renal metabolites of thyroxine and 3,5,3′-TRITh. Would these substances be intermediates leading to the iodothyroacetic acids by oxidative decarboxylation? All these substances have hormone activity. The triiodothyropyruvic acid isolated

from bile of rats treated with labelled 3,5,3′-TRITh has the same activity as thyroxine on amphibian metamorphosis (184). The acetic acid analogues of thyroxine and 3,5,3′-TRITh have antigoitrogenic activity similar to that of thyroxine itself, and 3,5,3′-triiodothyroacetic acid increases the BMR of normal rats in five days (186). The compound with four iodine atoms increases BMR in thyroidectomized rats 2 hr. after injection; the effect is gone after 4 hr. The dose given has to be strictly limited, since there is no increase with a larger quantity. Triiodothyroacetic acid produces characteristic changes in the feathers of thyroid deficient birds (187). However there is no significant increase in BMR when it is given to myxoedematous patients (188). The differences in behavior are difficult to explain by its rapid action alone.

There is another aspect of the problem which is of importance. The hypothesis representing thyroxine as a reservoir (189) producing 3,5,3′-TRITh by specific dehalogenation in the cells is incompatible with the fact that tetraiodothyroacetic acid is active immediately. It is hard to suppose the metabolic chain to be as follows: thyroxine, 3,5,3′-TRITh, later 3,5,3′-triiodothyropyruvic acid, triiodothyroacetic acid, and then tetraiodothyroacetic acid. It can be considered that if the acetic acid analogues of iodothyronines are the active tissue forms, they would each be derived from the corresponding thyroid hormones.

*Actions of synthetic thyroxine analogues.*—The natural substances having thyroid activity are all iodinated derivatives of thyronine which itself seems to be quite inactive. A large number of compounds have been synthesized in order to find out whether the entire thyronine structure is necessary for hormone action or whether in changing it there is simply a variation in the degree of hormone activity. Several very complete papers have appeared on this subject (190, 191, 192). Only those recently studied substances which have not appeared in the previous reviews will be mentioned here. Research has been done to establish whether iodine can be replaced by other halogens. Mussett & Pitt-Rivers (193) studied the antithyroid activity of trihalogenated derivatives. 3,5,3′-tribromothyronine is about 12 times less active than the corresponding triiodo compound, but it is still 100 times more active than 3,5,3′,5′-tetrabromothyronine. Comparing the bromothyronines shows that 3,5-diiodo-3′-bromothyronine is more active than 3,5-dibromo-3′-iodothyronine, which in turn is 30 per cent more active than thyroxine. The relative activities of these compounds show the importance of a single halogen substituent ortho to the phenolic hydroxyl group.

Roche *et al.* (183) studied the antigoitrogenic activity of various iodothyronines and iodo-desamino-thyronines. DL-3,3′-DITh is 82 per cent as active as DL-thyroxine, while 3,5-DITh is practically inactive. DL-3,3′,5′-TRITh has a rather low activity; its activity is only 5 per cent that of DL-thyroxine, while DL-3,5,3′-TRITh is 5 to 10 times more active than DL-thyroxine. 3,5,3′-triiodothyropropionic acid is as active as DL-thyroxine as an antigoitrogenic substance; 3,5,3′,5′-tetraiodothyropropionic acid has 75

per cent of the activity of thyroxine. The influence of several thyroxine analogues on batracian metamorphosis has been published. Bruice, Winzler & Kharasch (194) studied the activity of 22 substances. Among these substances 3,5-diiodo-3′,5′-dimethylthyronine was 1½ times more active than thyroxine, 3.5-diiodothyropropionic acid had 80 per cent of thyroxine activity, and 3,5,3′,5′-tetraiodothyroaniline was four times more active. But a particularly interesting substance was 3,5,3′,5′-tetraiododesaminothyronine, since it was 130 times more active than DL-thyroxines. Recently Roche and co-workers (184) made similar studies on fifteen compounds, of which five were iodothyronines, eight were tri- and tetraiodinated thyroacids with different side chains, and two were nitro derivatives. The results show that all substances having three iodine atoms in position 3,5,3′ are definitely more active than the corresponding tetraiodinated substances. The thyronine molecule does not become active either by one iodine atom in position 3′, or by two iodine atoms in positions 3,5 or in positions 3′,5′. The importance of the side chain is shown in the unusual efficiency of the propionic and acrylic acid derivatives. The tri- and tetranitroderivatives of thyronine have no action on metamorphosis. There are large differences in activity depending on the test used, especially for the iodothyropropionic acids. No hypothesis has been given to explain these differences. Perhaps the fact that D-thyroxine has 1/10 the activity of the natural L form might be explained by its transformation in the liver to tetraiodothyropyruvic acid which has ⅓ the activity of DL-thyroxine on batracian metamorphosis.

The point of view of Frieden & Winzler (195) should be modified, as far as it concerns certain structural conditions required for the actions of thyroxine and its analogues. For these authors substitution of halogen atoms in position 3 and 5 would be required and substitution in position 5′ and 3′ would greatly enhance activity. Actually it appears this is not so. A single substituent in position 3 together with a second in position 3′ is necessary. With a third substituent there is a great increase in activity if it is on position 5 and somewhat less if on position 5′. The examples of 3,3′-DITh, 3,5,3′-TRITh and 3,5′,3′-TRITh are demonstrative. The loss of activity by substitution in position 5′ is also clear, if one compares the efficiency of 3,5,3′-TRITh to that of thyroxine. It thus seems that among all the compounds tested, 3,5,3′-TRITh has the structure best suited for thyroid hormone activity.

## CONCLUDING REMARKS

The physiology and biochemistry of the thyroid tends more and more toward the study of cellular metabolism of the hormones. The fact that certain hormone metabolites are as active as the original substances, and that others may be even more active, can lead one to suppose that the hormone activity is attributable not only to products of thyroid secretion, but also to substances resulting from cellular transformation. This means that many new problems arise. It may be pointed out that the diversity of hormones

does not correspond, as in the case of the steroids, to quantitative differences in their effect, for instance one being more active on the oxygen consumption, another on the heart rate. Previously it was admitted that biological activities were directly connected to the hormones, but the problem seems more complicated now, as it is necessary to suppose that their metabolites are the active forms. Then the idea of cellular utilization of products of thyroid secretion constitutes the main goal of future work.

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# EFFECTS OF RADIATION ON MAMMALS[1,2]

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## INTRODUCTION

Since the last review in this journal on the biological effects of radiation (1), there have been more than 500 additional publications. It is impractical to review the entire area; therefore, the authors have selected those which were thought to be of most interest to physiologists and radiobiologists, and in which work is continuing. It has been necessary to omit many papers, and others have not been given the attention they deserve. The problems concerned with the physical and chemical effects of radiation on pure and simplified systems will not be considered, except where they apply directly to the mammalian effects under consideration. Furthermore, reports from the Atomic Energy Commission contract laboratories and the Department of Defense will be considered only when they bear upon one of the major topics under review, since most are eventually published in established scientific literature. In the past two years a series of books and review articles has been published. Belated publication of the results of the World War II research on effects of radiation continues (2, 3); however, the material is too voluminous to cover in the allotted space. In 1946–47 these volumes would have been of great interest but are now somewhat anticlimactic. For a general survey of the physical, chemical, and biologic effects of radiation, including an interesting discussion of mammalian effects, the provocative dissertation of Bacq & Alexander (4) is pertinent. The second edition of Lea's book (5) on radiation effects on living cells will make this fundamental work more available. The trend in thought and emphasis on biologic effects of radiation, particularly in Europe, can be obtained from the summary of the Interna-

[1] This survey covers the period of June, 1953 to July, 1955. Henceforth the entire field of effects of radiations on mammals will no longer be covered in a single chapter because the literature is too extensive for effective coverage. Instead, special topics in the field will be given more intensive coverage. A chapter on "Modification of Radiation Effects by Chemical Means and by Post-irradiation Restoration of Tissues" by the authors of the present chapter will appear in Volume 19 (1957) as the first of a series of chapters on special topics in the field.

[2] The following abbreviations have been used in this chapter: ACTH (adrenocorticotropin); LET (linear energy transfer); RBE (relative biological effectiveness); rem (roentgen equivalent, man); rep (roentgen equivalent, physical); RNA (ribonucleic acid); DNA (deoxyribonucleic acid); MCA (methylcholanthrene); ATP (adenosinetriphosphate); TSH (thyroid-stimulating hormone); G.I. (gastrointestinal); KV (kilovolts); KVP (kilovolt peak); EEG (electroencephalograph); WBR (whole body radiation); RQ (respiratory quotient); DPN (diphosphopyridine nucleotide); DPNH (reduced diphosphopyridine nucleotide); RE (reticuloendothelial); RES (reticuloendothelial system); STH (somatotrophic hormone); A.G. (albumin-globulin).

tional Radiobiology Meeting held in Aarhus, Denmark, in 1953 (6), and from the report of the 1955 Radiobiology Symposium held at Liege (7). The review by Ord & Stocken on the biochemical effects of radiation (8) is most useful, as is the two-volume book edited by Hollaender (9). Cellular radiobiology, with emphasis on the cytochemical and chromosomal effects (10) has been reviewed. Carcinogenesis and histopathologic effects were considered by Furth & Upton (11), and by Furth & Lorenz [in (9), Chapt. 18]. The most recent review of Lorenz & Congdon (12), among other topics, included some discussion of linear energy transfer (LET), and its relation to biologic effect in small living objects. Since relative biological effectiveness (RBE) is of considerable importance in trying to establish permissible doses of various ionizing radiations, and since it aids in understanding the fundamental effect of radiation on diverse tissues at a subcellular level, mammalian RBE's will be considered in some detail in this review.

## RELATIVE BIOLOGICAL EFFECTIVENESS (RBE)

Several articles and reviews on the general subject of the RBE of different ionizing radiations have appeared (13 to 16) and an increasing number of papers dealing with RBE in mammals has been published. The RBE of two radiations can be defined as the inverse ratio of the doses of the two radiations required to produce a standard amount of a given biological effect.[3] Usually, a radiation of unknown RBE is compared to a standard (x- or $\gamma$ radiation), and the RBE is numerically equal to the dose in rem for a given effect divided by the dose in rep to produce the same degree of effect (13 to 16). The rem is usually defined as that dose of any radiation which produces a biological effect equal to that produced by 1 roentgen of x- or $\gamma$ radiation, and has been more specifically defined by Failla (17) as being "the quantity of any ionizing radiation such that the energy imparted to a biological system . . . per gram of living matter by the ionizing particles present in the locus of interest, has the same biological effectiveness as 1 rep of X-radiation."

The term RBE has been given a variety of meanings by different authors, depending principally on what is meant by "dose" of radiation, and the meanings fall into two general categories. In one category, the dose of radiation is physically measured and accurately known throughout the biological material under study, and thus variations in the degree of biological effect reflect differences in the properties of the radiations themselves, resulting in differences in mechanism of energy absorption and of biological effect. This use of the term is implied in Failla's definition, and it has been used principally in work with microorganisms in which the energy absorbed per gram of material, or the physically measured dose of radiation, is accurately known. With microorganisms, particularly by using the single track segment tech-

[3] Another possible definition, the ratio of degrees of biological effect produced by dentical doses of different radiations, is of very limited usefulness.

nique, it has been possible to correlate differences in RBE with differences in LET[4] along the path of the ionizing particle, from which conclusions relating to the fundamental biological effects of radiation can be drawn.

A second category of usage of the term RBE involves situations where the dose either is only poorly known, not known at all, or measured with "biological dosimeters" rather than in physical terms. The term has been used in comparing the effects of different $\alpha$ emitters deposited in bone, in which the microscopic distribution of the different radiations is known to differ widely and where radiation dose could not be estimated. In such situations, fundamental interpretations of the apparent RBE, such as are possible in the first category, cannot be made. This use of the term is employed principally in work obviously utilitarian or practical, as in considerations of maximum permissible exposure or of radiation therapy. It is a most useful term when applied in this fashion, provided its limitations and necessary qualifications are recognized.

Although, with improved dosimetry, some work with RBE in mammals can now approach the standards for Category 1, the bulk of mammalian work must be considered in varying degrees, to be in Category 2. Many authors whose work belongs in Category 2 have attempted to interpret their results as belonging to Category 1. Some of the difficulties of obtaining RBE values in the mammal, interpretable as Category 1, are outlined below [see Boag (13)].

A difficulty common to all RBE determinations in any biological system is the complete dependency of the obtained value on the system and methods employed in its determination. The RBE may depend upon the species employed, the age, the particular biological endpoint observed, the degree of biological response compared (e.g., the RBE for 20 per cent mortality may be different than for 80 per cent mortality), the diet, temperature, oxygen tension, water content, pH, and dose rate or total time of exposure. The RBE may be quite different for very similar tissues in the same animal. Thus, it is hazardous to extrapolate RBE's beyond the particular situation employed. In the mammal, two principal additional difficulties appear. Obviously, in a complex organism a variety of effects are produced by radiation which may be elicited to different degrees by different radiations and all of which may contribute to the particular biological endpoint observed (the abscopal effect, for example). The degree of interaction among these more simple effects cannot be estimated, and the net effects may be qualitatively different. A second major problem lies in the difficulty of determining physically the dose at the "locus of interest" in the mammal, even if one is confident that the locus of interest is known. In large tissue masses it is frequently not feasible to obtain uniform dosage distribution because of depth dose, or differential

[4] LET, rather than specific ionization, is preferred, since use of the latter term involves unwarranted extrapolations to liquids or solids from data obtained with gases.

absorption in tissues. For example, with slow-neutron irradiation of the mouse, the exit dose may be as low as 20 per cent of the entrance dose. Similar fall-off may occur with unilateral irradiation of the dog with 250 KVP radiation. With neutron radiation, it is not possible to measure energy absorbed per gram, or dose, except with highly specialized apparatus not generally available. Confusion has resulted from the use of different values of ergs per gram for the rep, and from the use of roentgens measured free in air in some cases and in the biological specimen in others. Considerable confusion has also arisen because a "standard" radiation for comparison has not been sufficiently well-defined. Thus, some authors report RBE's in terms of 250 KVP x-rays as a standard, while others have used $\gamma$ radiation. For most biological endpoints the ratio of effectiveness of these two "standards" differs considerably from 1. Usually with neutrons, some degree of effect produced by inherent $\gamma$ radiation must be subtracted from the total effect to obtain an RBE for neutrons, and the degree of additivity of the two radiations frequently is not known. If acceptably uniform dosage throughout the tissue mass is obtained the microscopic distribution of dosage usually is not known. In the mammal it is rarely possible to obtain energy deposition in sharply demarcated intervals of LET as can be done with microorganisms. Even though one could begin with a monoenergic source, degradation in air and in tissue is rapid (18). Invariably, a wide spread of LET values results for the particles delivering the dose to the tissues. And, of course, the LET varies markedly along the path of the particle in the tissue. One must therefore be content with an "average" LET value, which may only poorly represent the spectrum of energy transfer within the tissue. Thus, the use of the term "RBE" in the mammal obviously may not conform to the strict definitions implied in the term, and conclusions from such data relative to fundamental differences in mechanisms of action of different radiations can be drawn only with extreme caution.

*X- and $\gamma$ radiation.*—The RBE of x- and $\gamma$ radiations of different energies has received considerable attention. Grahn *et al.* (19), in carefully controlled mortality studies in hybrid $BAf_1$ mice, found the relative effectiveness of 250 KVP, 135 KVP, and 80 KVP x-rays to be 1.0, 0.96, and 0.78, respectively. Doses were measured free in air, and the decreased effectiveness of the lower KVP radiation was presumably related principally to decreased penetration. Cronkite *et al.* (20) found 2,000 KVP x-radiation to be approximately 0.8 times as efficient as 250 KVP x-rays for acute lethality in mice. Upton *et al.* (21) found $\gamma$ rays from $Co^{60}$ to be 0.72 times as effective as 250 KVP rays for acute lethality in mice. The $LD_{50}$ values for mice exposed to $Co^{60}$ $\gamma$ radiation by Clark *et al.* (22) were considerably higher than those reported by others for x-rays in the same strain of mouse, indicating a reduced relative efficiency of the $\gamma$ radiation.

Storer *et al.* (23) determined the effectiveness of thulium$^{170}$ radiation, as compared with 200 KVP x-rays, and obtained a value of 0.54, using spleen-thymus atrophy in the mouse as the endpoint (thulium$^{170}$ radiation has a

theoretical maximum energy of 970 kv. with peak intensities at 52 and 84 kv.). Cronkite & Brecher (24) reported an $LD_{50}$ of 725 r for rats exposed to $Co^{60}$ $\gamma$ radiation, as compared to a value of 600 r for 250 KVP x-radiation. Harris is quoted (see 15) as stating that the RBE of $Co^{60}$ $\gamma$ radiation, compared to 250 KVP x-radiation, is 1 if spleen and thymus weight loss is used as the biological indicator. This again emphasizes that the RBE is highly dependent on the biological system under study.

The Los Alamos group compared the effects of 4 Mev $\gamma$ radiation from the Los Alamos reactor to those of 250 KVP x-rays, and the results were summarized by Harris (25). The values obtained for the various biological endpoints employed in mice were: 30-day mortality, 0.53; spleen weight loss, 0.60; thymus weight loss, 0.80. A value of 0.68 was obtained using iron uptake by rat erythrocytes as the endpoint.

The effects of x-rays from a 22.5 Mev betatron have been compared with those of 180 KVP x-rays, using acute mortality in mice as the endpoint (26). The RBE was found to vary with dose, and was 0.87 at 500 r and 0.42 at 800 r. Haas *et al.* (27) compared the effectiveness of 19 Mev electrons and 23 Mev x-rays to those of 400 kv. x-rays, using rat mortality as an endpoint, and obtained values of 0.78 and 0.80, respectively. Dittrich *et al.* (28) found 4 Mev electrons and x-rays to be equivalent in producing hematological changes in rats given total-body radiation.

Bane *et al.* (29) obtained an RBE of 1 for 22.5 Mev x-rays compared to 180 or 250 KVP x-rays, using reduction in mouse testis weight as the criterion of effect. Fritz-Niggli (30) reported 31 Mev radiation to be less effective than 180 KVP x-radiation. Woodard (31), comparing the loss of alkaline phosphatase in mouse bone following exposure to x-rays of peak energies of 100, 180, 1000, and 22,000 KVP, found relative efficiencies of 0.96, 1.00, 0.76, and 0.88. This was on the basis of incident dose. On the basis of absorbed energy at the locus of interest, the author felt that the RBE values were unity. Barth *et al.* (32) used lethality in rats as the biological endpoint and found the ratio of effectiveness of 15 Mev electrons and 190 KVP x-rays to be approximately 0.8. Ting *et al.* (33) used tumor regression in mice to compare the effects of 23.5 Mev x-rays to those of 200 KVP x-rays and obtained values of 0.87 for an adenocarcinoma, and 0.81 for a sarcoma. Haas *et al.* (27) found 19 Mev electrons and 23 Mev x-rays to be less effective than 400 kv. x-rays in producing damage to human skin by ratios of 0.78 and 0.80. Wachsmann (34) determined that 1.5 times or more of radium $\gamma$ radiation was necessary to elicit the same human skin reaction as with 45 to 200 kv. x-rays.

The relative effectiveness of different energy x-radiations has been studied in large animals. Grahn *et al.* (19) irradiated rabbits through multiple ports to achieve as uniform dose distribution as possible. Although the data were complicated by deaths within the first three days, the $LD_{50}$ again rose sharply where penetrability fell off rapidly at 100 KVP (see under mice above). The $LD_{50}$ values for 250 KVP, 100 KVP, and 80 KVP were 807, 1327, and 1887 r, respectively (doses measured in air). Greenfield *et al.* (35) found no difference

in lethality in rabbits irradiated by two opposing 250 KVP x-ray machines, or by one separately. Bond *et al.* (36), using bilateral irradiation in dogs to achieve a more uniform dose throughout the animals, found 250 KVP, 1000 KVP, and 2000 KVP x-radiations to be essentially identical in their effects.

Rust *et al.* (37) have exposed swine and burros to $\gamma$ radiation from 19 separate sources distributed over a plane surface. For $\gamma$ radiation from $Ta^{182}$, they reported $LD_{50}$ values in burros of 651 r for dose rates of 18 to 23 r/hr. For $\gamma$ radiation from $Co^{60}$, $LD_{50}$ values of 784 r for the burro and 618 r for swine were reported (38). The dose rate was 51 r/hr., and all doses reported were measured in air. The dose at the median sagittal plane of the burro was approximately 70 per cent of the skin dose as determined by phantom measurements. The authors pointed out the difficulties of comparing their data with previous results on large animals because of the size and species of animal used, the particular geometry of exposure employed, the types of radiation used, and the relatively low dose rate. This type of data assumes particular importance, since it simulates much more closely than do conventional sources the type of exposure to $\gamma$ radiation that results from fall-out (39).

All large animal studies have served to emphasize the importance of depth dose and of dose distribution throughout the body in quantifying the response to radiation. In all species, the apparent effectiveness drops off at the point where, because of low energy or unfavorable geometry, the distribution of dose throughout the body departs significantly from uniformity (19). Under these conditions, the results cannot be interpreted in terms of varying LET. A condition of essentially partial body radiation exists and the relatively shielded tissue undoubtedly alters the effects obtained. With total-body irradiation of mice, there appears to be little doubt that, for most endpoints, the effectiveness decreases as energy is increased from approximately 250 KVP to 2000 KVP and higher. Over this same range in the dog, with essentially uniform total-body irradiation, there appears to be little, if any, change in effectiveness. Unpublished data of the authors in swine indicate similarly little, if any, change in effectiveness. This raises the question of whether the changing effectiveness in mice should be ascribed to changing LET or whether the explanation should be sought elsewhere. It is possible that, although the gross distribution of dose throughout the animal mass may be the same, local or microscopic differences resulting from skeletal influences may be marked. Absorption in bone is, of course, dependent upon energy. Some hint of such an explanation is seen in Woodard's work on bone alkaline phosphatase. Her data indicated that, while in terms of air dose, the RBE varies with x-ray energy, in terms of dose at the locus of interest, the RBE may be unity.

The effectiveness of the initial $\gamma$ radiation from the atomic bomb has been determined in mortality studies using mice (20) and swine (40). The $LD_{50}$ for $LAf_1$ mice was found to be 680 r for the bomb $\gamma$ rays, as compared to 650 r for 250 KVP x-radiation; thus, the RBE was essentially unity. In view of the very high energy of the primary beam, it might be expected that the RBE

would be less than unity. However, as shown by Gates & Eisenhauer (41), the beam at distances of primary biological interest would be expected to be degraded, and a large portion of the dose probably is delivered by photons of 200 kev or less. The $LD_{50}$ for swine was found to be 230 r, and the authors stated that the data agreed with previous 2000 KVP x-irradiation studies. From phantom measurement studies using a point source versus an essentially planar source, such as that encountered in a fall-out field, it would be anticipated that fall-out radiation, per roentgen measured in air, would be more effective than the identical radiation from a point source (39).

Attempts have been made (42, 43) to determine the RBE of $\beta$ rays by administering tritium (a pure $\beta$ emitter) as $T_2O$, which has the same distribution as body water. Using spleen- and thymus weight loss in mice as the biological indicator, these authors found RBE values of 1.4 (43). By using uptake of radioactive iron in rats, a value of 1.6 was obtained (42).

*Fast neutrons.*—Neutron physics of interest to the biologist have been presented (44), and the problems involved in neutron dosimetry have been reviewed recently (45). The conversion factor for "$n$" units to rep has been checked by Rossi (46), using tissue-equivalent chambers. Using cyclotron neutrons of approximately 9.5 Mev average energy, he obtained rep/$n$ conversion factors of from 1.76 to 2.00 for a 25 r ionization chamber, and 1.86 to 2.11 for a 100 r chamber. Fast fission neutrons from the exposure chamber used by Clark *et al.* (22) yielded values of 1.77 to 2.05 for the 25 r chamber and 1.61 to 2.06 for the 100 r chamber. Boag (13) re-evaluated the $n$ to rep conversion factor, particularly in light of Rossi's data, and felt that a value of 2.0 for fast neutrons should be used. Hurst has reported on an absolute tissue dosimeter for fast neutrons (47) and Rossi & Rosenzweig (48) have reported a method of measuring neutron dose as a function of LET. Depth-flux and depth-dose measurements in tissue equivalent media have been described (49, 50). The above-mentioned articles should be consulted for an understanding of the mechanisms of absorption of energy in tissue from neutrons and how they differ from those with x-radiation.

Several groups have reported on the RBE of fast neutrons in mice. Harris *et al.* (51) exposed CF 1 mice to a monochromatic 14 Mev neutron beam generated by means of a Cockroft-Walton accelerator in which a tritium target was bombarded with deuterons. Using spleen and thymus weight loss as the biological indicator, one roentgen was found to be equivalent to $1\times10^8$ $n$/cm$^2$. Flux values were converted to energy absorbed in tissue using first collision rep curves calculated from cross-section data for the principal elements in tissue. This method has been presented in some detail by Tochilin *et al.* (49). The RBE was found to be 1.6. Carter *et al.* (52) determined the RBE for fast neutrons in mice, using a variety of biological endpoints. Fast neutrons of mean energy of approximately 9 Mev were generated in the 60-in. Crocker Laboratory cyclotron, using the Be ($D$, $n$) B reaction. Flux was measured with sulfur threshold detectors, from which the rep dose was calculated (49). The degree of $\gamma$ contamination was measured and found to be less than 10

per cent. The RBE values obtained were as follows: spleen weight loss, 1.7; thymus weight loss, 1.5; small intestine weight loss, 2.3; 30-day mortality, 1.6. Upton *et al.* (53) exposed RF mice to fast neutrons obtained by bombarding an internal target with 22 Mev deuterons in the Oak Ridge 86-in. cyclotron. Dosimetry was accomplished in a variety of ways (54). The total flux was measured, from which total rep was calculated. The values thus obtained agreed within 10 per cent with measurements obtained from a proportional neutron counter. The degree of $\gamma$ contamination was determined by means of film badge measurements, and the total dose (neutron plus gamma) agreed well with readings obtained with the Failla-Rossi tissue equivalent chambers. The RBE value for fast neutrons was found to be 2.01 compared to $Co^{60}$ $\gamma$ radiation (1.46 compared to 250 KVP x-radiation), using 30-day mortality in the mouse as the biological indicator. Riley *et al.* (55, 56) have used 30-day mortality in mice to estimate the RBE of neutrons from a 37-in. cyclotron (average energy 3 to 4 Mev) and from a 60-in. cyclotron (average energy reported to be 15 to 16 Mev for one beam; 7 to 8 Mev for a second beam). Doses originally were expressed in terms of the "*n*" unit (55); however, the beams have now been calibrated in terms of the Failla-Rossi tissue equivalent chambers. The RBE was found to vary inversely with the energy of the neutrons; from 2.8 for neutrons of about 1 Mev to 1.2 for neutrons in the 7 to 12 Mev range, as compared to 200 KVP x-rays. It is not clear if the gamma component of the beams was measured and subtracted, a procedure that would raise the RBE. Clark *et al.* (22) have conducted experiments using fast fission neutrons from a fission plate. The neutron spectrum employed should not differ greatly from that reported by Watt (57). Dosimetry was accomplished with a 100 r capacity ionization chamber calibrated in terms of the Failla-Rossi tissue-equivalent chambers. Using acute mortality in CF1 female mice as a criterion, the effectiveness of the neutrons relative to $Co^{60}$ $\gamma$ radiation was found to be 4.4 (approximately 3.2 if compared to 250 KVP x-rays). The same RBE was obtained, using spleen-weight loss as the criterion of effect (58).

Considering the widely-separated laboratories and the widely-diversified procedures and dosimetry, the values recently reported for the RBE of fast neutrons are remarkably consistent and considerably lower than those appearing in the older literature. For high energy neutrons, from 1 to 2 Mev to in excess of 10 Mev, the RBE appears to be fairly uniform and to be between 1 and 3 (compared to 250 KVP x-rays) for essentially all acute endpoints studied.

The relatively shortened survival time of mice following total-body irradiation with neutrons, first observed by Hagen & Zirkle in 1946 (59), has been abundantly confirmed by later investigators (52, 60). The difference in survival time has been interpreted as reflecting a difference in mechanism of death (52), and recent studies on the lack of complete additivity of neutron and $\gamma$-ray effects support this conclusion (60). The short survival time has been associated with the sequelae of early gut damage; longer survival

times with the sequelae of the later bone marrow damage (61). On this basis, it would not be possible to strictly compare 30-day mortality from neutron and $\gamma$ radiation. The shortened survival time in mice can be produced with x- or $\gamma$ radiation; however, the doses required are much higher than for the later deaths (6-day $LD_{50}$ in mice is nearly twice the 30-day $LD_{50}$). Thus, if the same mechanism of mortality is compared with the two radiations, the RBE would be raised by a factor of almost 2.

Carter *et al.* (52) found the RBE of fast neutrons for loss of weight of the small intestine of mice to be higher than for spleen-thymus weight loss or for mortality. The short survival time following fast neutron irradiation would indicate that the bowel and bone marrow have relatively different sensitivities to x- and neutron radiation, or that these tissues received different relative doses of these two radiations. Reduced dose to the bone and the adjacent cell layer with neutrons has been suggested as an explanation because of the relatively low hydrogen content of bone (52). Tait (62), however, calculated the energy deposition from neutrons in bone and the adjacent soft tissue, and concluded that there was no significant difference.

Clark *et al.* (22) have observed that while the $LD_{50}$ for mice increases with $\gamma$ radiation if the total dose is given over 24 hr. as opposed to 90 min., no such effect is obtained with fast neutron exposure. The explanation for this phenomenon is not apparent; however, it may help to explain some of the high RBE values reported by early workers who were forced, because of limitations of exposure apparatus, to employ slow dose rates.

Data on biological materials exposed to fast neutrons from the atomic bomb have been published (63). Because of uncertainties in dosimetry, it is difficult to calculate RBE's for the fast neutrons from atomic weapons with any degree of certainty. The values of 7 to 8 for reduction of spleen-thymus weight, and of 3.9 for reduction of absolute peripheral lymphocyte count [Carter *et al.* (63)] are maximal values and are probably too high. The data emphasize again, however, that the RBE, even for similar tissues, can vary considerably in the same animal.

Russell (63), using dominant lethality in the offspring of male mice exposed in lead hemispheres to fast neutron radiation from an atomic detonation, concluded that bomb neutrons and those produced in a cyclotron were equally effective and that the bomb neutrons were between 6.4 and 9.4 times as effective as x-rays. Dosimetry was accomplished with tissue equivalent chambers, and the limits of the RBE are those felt by the authors to represent the limits of uncertainty in the dose measurements, particularly in the gamma contribution.

Bond *et al.* (36) exposed dogs to fast neutrons with an average energy of approximately 9 Mev. Bilateral irradiation was used (one-half of the total dose to each side), and this resulted in a more uniform dose distribution throughout the animals. Dosimetry was done as described above (49). The $LD_{50}$ doses, expressed as dose to the midline of the animals as determined from phantom measurements, were 289 rep for neutrons and 252 rep for

250 KVP x-rays (RBE of 0.8). The biological effects were qualitatively identical to those produced by high energy x-radiation, and the shortened survival time observed in mice following total-body neutron irradiation was not observed in dogs.

*Slow neutrons.*—The effectiveness of thermal neutrons for mortality in mice was investigated by Brennan *et al.* (64) in a thorough and systematic study, which indicated clearly the mechanism of action of slow neutrons in tissue and the problems of dosimetry. He determined the dose contribution from the inherent $\gamma$ radiation (4 Mev) from the Los Alamos homogeneous reactor, in the mouse exposure apparatus used, to be 15 per cent of the total rem dose. Correcting for this (using an RBE of 0.53 for the 4 Mev $\gamma$ rays), for the inter-mouse $\gamma$ irradiation from hydrogen capture $\gamma$ rays (measured as rem dose from the 2.2 Mev capture gammas), and also for the "sink" effect of the mice in reducing the thermal neutron flux, he determined that $1.37 \times 10^{10}$ $n/cm.^2$ were equivalent to 1 rem. He calculated the rep dose from the known interactions of thermal neutrons with tissue and determined, under his conditions of exposure, that 42 per cent of the total rep dose resulted from capture gammas from hydrogen, 54 per cent from recoil protons from nitrogen, and 4 per cent from heavy particles from boron. Using these values, the RBE for thermal neutrons was determined to be 1.70.[5] If the rem dose from capture $\gamma$ rays was subtracted, assuming an RBE of 0.8 for the $\gamma$ rays, the RBE for recoil protons and for the heavy particles from boron capture combined became 2.6. The validity of subtracting $\gamma$-ray effects from those due to slow neutrons was established in a later study (65) in which the complete additivity of x-ray and slow neutron effects for mortality in mice was demonstrated.

The RBE's of thermal neutrons for a wide variety of additional biological endpoints have been determined subsequently, by use of essentially the same approach and facilities employed by Brennan (64), and the results have been summarized (25, 66). The values reported are as follows for the mouse: spleen weight loss, 1.6; thymus weight loss, 1.7; inhibition of mitosis in the mouse ear, 1.7; and testicular atrophy, 1.3. In rats, values of 1.4 for acute lethality, and 1.1 for both small-intestinal atrophy and iron uptake were reported. All of these values depended upon the effectiveness of inherent 4 Mev $\gamma$ rays in the reactor relative to the 250 KVP standard radiation used, and the value for the RBE of thermal neutrons decreases as the effectiveness value used for the $\gamma$-rays increases. For some endpoints (mortality and spleen-thymus weight loss), the effectiveness was measured. Values were assumed for the remaining endpoints. Complete additivity of $\gamma$ and thermal neutron radiation was determined for mortality and assumed for the remaining endpoints. For the standard x-radiation, the free-in-air dose was used and was assumed to be equivalent to rep. If the 9 per cent backscatter under the conditions em-

[5] If this value is recalculated using 4 per cent nitrogen content of tissue, instead of 3 per cent, the RBE becomes 1.5.

ployed (64) is added to the air dose to obtain "tissue dose" or rep, the RBE values noted would increase by that amount.

For testicular atrophy in mice, the authors obtained values for the thermal neutron RBE as high as 2.5, if assumptions relative to depth distribution of dose in the testis, different from those used for other endpoints, were made. Thus, differences in the manner of arriving at the RBE can influence considerably the values obtained; however, it is unlikely that marked changes in the reported values are likely from these considerations. Most authors gave no evaluation of the accuracy of flux density measurements on which calculated dose and, thus, RBE depend, nor were depth-flux or depth-dose measurements usually included. Upton *et al.* (67) stated that the thermal neutron flux at the midline of a mouse under his conditions was 75 to 80 per cent of that at the surface. Thus, the median dose to the animal in many cases may be lower than that reported, and the RBE correspondingly higher by that amount, if the median dose or midline dose is used.

The thermal neutron flux equivalent to 1 r of x-radiation has been determined by the Los Alamos group to range for acute effects from $1.49\times10^{10}$ $n/cm.^2$ for uptake of $Fe^{59}$ by rat erythrocytes to $0.82\times10^{10}$ $n/cm.^2$ for testicular atrophy in mice (25). This compared with a value of $0.79\times10^{10}$ $n/cm.^2$ equivalent to 1 r, as determined in the Brookhaven reactor using the growth delay response in rats as the biological indicator (68). On the basis of acute lethality and hematological effects, Upton *et al.* (67) has calculated that $1.7\times10^{10}$ $n/cm.^2$ from the Oak Ridge reactor are equivalent to 1 rem. Most of these values were obtained using an RBE of approximately 0.6 in subtracting the effects attributable to the inherent 4 Mev reactor $\gamma$ radiation.

The relatively short survival time, well established for mice receiving total-body fast neutron irradiation, can also be seen from Brennan's data (64) for mice exposed to thermal neutrons. Reasoning that if the phenomenon of shortened survival time is associated with relatively greater neutron effect on the intestine it should be reflected in relatively greater damage to the intestine, Storer & Furchner (69) investigated the problem in rats using loss of gut weight as an endpoint. They compared the results with those obtained using the uptake of $Fe^{59}$ by the red cells as an index of effect. The RBE's for both endpoints were the same. It should be pointed out, however, that the early-death phenomenon was not at all pronounced in their rats. The ratio of 8-day to 30-day $LD_{50}$ for neutrons compared with that for x-rays, widely different in Brennan's data for mice exposed to slow neutrons (64), was the same for rats. This minimal tendency to early deaths in the rat was in keeping with unpublished data of Bond, indicating that the early death phenomenon with fast neutrons in the rat was markedly less pronounced than in the mouse. Carter *et al.* (52) found a relatively large RBE for fast-neutron irradiated mice, using intestinal atrophy in the mouse, and it would seem that similar studies on mice should be done using slow neutrons.

Bond *et al.* (70) have investigated in mice the apparent RBE of heavy

particles resulting from neutron capture by boron given intravenously prior to thermal neutron irradiation. Irradiation from boron capture and from reactor radiations was found to be completely additive. The RBE, using spleen-thymus weight loss as the biological criterion and comparing with $\gamma$-ray effects, was found to be 1.4. Boron is believed to distribute essentially as does body water. Thus, it is of interest to compare this value with that of 1.3 to 1.5 obtained for tritium (43), a pure $\beta$ emitter which is uniformly distributed throughout body water when given as $T_2O$.

Considering the various reported RBE values, a pattern develops in which the RBE for acute endpoints in the mouse appears to change from 1.0 for $\gamma$- or very high energy x-rays, approximately 1.5 for x-rays of lower energy, 2 to 2.5 for high energy neutrons (recoil protons), and higher for lower energy fast neutrons. From this, it is attractive to postulate that the RBE changes with changing LET. The relatively low RBE for $\alpha$ particles in the mouse could be taken as an indication that the RBE for the mouse passes through a maximum and becomes lower with very densely ionizing radiations, as has been demonstrated with microorganisms (71). However, the many difficulties and uncertainties in obtaining RBE values in a complex system such as the mammal, pointed out above, should be realized. With these myriad difficulties and limitations in mind, it would appear that the problem of RBE in mammals is in a data-collecting stage, in which attempts to interpret correlations of RBE with "average" LET in terms of basic mechanisms of radiation damage are premature.

## BIOLOGICAL EFFECTS OF RADIATION

### Cataractogenesis

The status of the problem of radiation-induced cataracts has been well summarized both in the reports of the annual National Research Council Conferences on radiation cataracts (72) and in a review article by Ham (73). Thus, only a few papers on RBE for this biological endpoint will be cited. Upton *et al.* (74) have demonstrated in mice exposed to x-radiation that cataractogenesis was a result of direct irradiation of the eye and resulted only if the eye was included in the radiation field. Similar results have been obtained by Alter & Leinfelder (75). Their data indicated further that, if only a portion of the lens was irradiated, cataracts appeared only in that section. The equatorial region of the lens was found to be most sensitive in this regard. McDonald *et al.* (76), using radon, $Sr^{90}$ and $-Y^{90}$, to irradiate the eye of rabbits and human beings, found the threshold for cataract formation to be lowest over the limbus. Such irradiation resulted in equatorial opacities and vacuoles which migrated posteriorly.

Riley *et al.* (55, 56) have exposed mice to fast neutrons of 4 Mev average energy and to 200 KVP x-rays, and obtained an RBE of 4 for mice given a single exposure. With three exposures one week apart, the RBE rose to 6; however, further fractionation did not further increase the RBE. (The authors stated that the true values may be higher by a factor of 1.5 because of

an incompletely evaluated gamma component of the beam.) Cogan *et al.* (77) exposed rabbits to 14 Mev and to fast fission neutrons. They determined that $5\times10^{10}$ $n$/cm.$^2$ of 14 Mev neutrons or $9\times10^{10}$ $n$/cm.$^2$ of fast fission neutrons were equivalent to 500 r of x-rays in terms of cataract production. Using Harris's conversion factor of $1.54\times10^8$ 14 Mev neutrons equivalent to 1 rep, they obtained an RBE value of 3.2 for this endpoint. A similar conversion factor for the fast fission spectrum is not available. Moses *et al.* (78) irradiated the eyes of rabbits locally with fast neutrons of 6 to 8 Mev average energy and determined that the threshold for cataract induction in rabbits was considerably lower than in dogs. The lens of the mouse appeared to be particularly susceptible to radiation-induced cataract formation (67).

Christenberry & Furth (79) exposed mice to thermal neutrons and determined the RBE for cataract production to be approximately four times that for acute effects. Storer *et al.* (see 25), using the same endpoint in CF 1 mice, obtained an RBE of 10.2.

It is definitely established that the RBE for cataract production resulting from either thermal or fast neutron irradiation is several times higher than for acute effects, such as 30-day lethality, spleen-thymus weight loss, etc. It is of considerable interest, however, that the RBE for late effects, other than that of cataract production, does not appear to differ from the RBE for acute effects. Thus, Storer *et al.* (66) found the RBE for 30-week mortality in mice to be identical to that for 30 days. Upton *et al.* (67) found the RBE in mice for carcinogenesis and for reduction in life span to be identical to those for acute effects.

## Carcinogenesis

Ordinarily, one might not consider carcinogenesis an appropriate subject for a physiological review. However, much of the experimental work that is now being reported emphasizes that there are important influences of remote tissues upon normal growth; thus, by studying development of neoplasia, additional light may be thrown on fundamental growth control processes.

Heretofore, with the exception of a possible statistical increase of leukemia in radiologists [reviewed in (11)], radiation-induced leukemia has been an animal phenomenon. Radiation has not been shown to be a leukemogenic agent in the guinea pig (80). Survivors from the atomic bombs at Hiroshima and Nagasaki, who were close enough to the hypocenter to have received radiation in the potentially lethal range, had a significantly greater incidence of leukemia than did survivors in the group outside the range of $\gamma$ radiation (81). Those who had severe, acute radiation illness have shown the highest incidence of leukemia. The leukemia has been predominantly myeloid and, to a lesser extent, monocytic (82). This is in contrast to radiation-induced leukemia of animals, which usually has been lymphoid. In the preclinical state of human leukemia a low alkaline phosphatase of separated leukocytes was found, along with a neutrophilic leukocytosis and the presence of increased numbers of basophils (83). The authors suggest that radiation-

induced chronic myelogenous leukemia is attributable to a loss of growth regulating factors (81). Acute leukemia has been observed following x-ray and radioiodine treatment of thyroid carcinoma (84).[6]

Tumors can be induced by direct action of one or more carcinogenic agents on the appropriate tissues. However, the evidence for an abscopal effect[7] of radiation on development of tumors in animals (e.g., ovarian, lung, mammary, and leukemias) is known (11). An abscopal effect in man was implied in the deductions on leukemogenesis in Japanese survivors from the atomic bomb (81). Leukemogenesis by ionizing radiations was analyzed by Furth & Upton (85). All types of ionizing radiation are leukemogenic. Single and repeated exposures can produce leukemia and repeated, properly-spaced exposures result in more rapid induction and a higher incidence. The induction rate increases with the dose. Physiologic and genetic factors markedly influence the induction of leukemia in mice. Androgens and cortisone inhibit, and estrogens enhance the induction of lymphoid leukemia. Cortisone, however, does not inhibit production of myeloid leukemia (86). Shielding of the normal spleen, which contains all types of hematopoietic tissues, protects against lymphoma induction in $C_{57}BL$ mice (87), as does injection of bone marrow suspensions (88). Kroning & Sigmund (89), using relatively high doses, showed that the incidence of lymphomas is decreased by fractionation.

Kaplan *et al.* (reviewed in 1, 11, 12, 85) had previously shown that thymectomy prevents lymphoma induction and that local thymic irradiation is ineffective in producing the tumors (90). Subsequently they showed that shielding and injections of marrow suspensions accelerates the regeneration of thymic tissue and that concommitantly thymic lymphoid tumors are inhibited (90). Recent studies (91) have shown that lymphoid tumors develop in nonirradiated thymic tissue grafted into thymectomized irradiated mice. This observation would appear to establish the development of a cancer in a tissue not exposed to the carcinogenic agent. However, a co-carcinogenic effect of trauma and necrosis in the graft may have a contributory effect, and cell migration to the transplanted tissue cannot be ruled out absolutely. The most recent studies of Kaplan *et al.* (92) show a definite time dependence for protection against induction of leukemia in mice by injections of homologous bone marrow. Lymphoid tumor induction is maximally inhibited when injections are made $1\frac{1}{2}$ hr. after the last x-ray dose. "Inhibition decreased in linear relation to log time and was essentially lost after a delay of about 16 days" (92). Females also developed a higher incidence of tumors at all treatment intervals. In other studies Weymouth *et al.* (93) studied ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in thymic cells of thigh-shielded and nonshielded, irradiated mice. It was

[6] The development of myelogenous leukemia in one patient undergoing similar therapy has been observed in the Medical Department at the Brookhaven National Laboratory.

[7] Abscopal effect is defined later in the text; see page 506.

found that DNA levels per thymic cell do not vary with age or treatment group and RNA remains constant with time in the control group, but RNA increases 1 to 5 days after irradiation with a prompt return to normal in the thigh-shielded group. The RNA per cell and RNA/DNA ratio remain markedly elevated over the entire course of treatment in the unshielded irradiated group. The authors suggest that the sustained elevation of RNA may be related to tumor induction, since shielding prevents induction and results in a rapid return of RNA to normal.

It is quite evident that leukemogenesis by ionizing radiations and the interactions between normal and irradiated hemopoietic tissues and endocrines, which are proved to influence induction, are most intricate. On the basis of experiments reported to date, it is not possible to determine whether the nonirradiated hemopoietic tissues supply a cellular or humoral factor that inhibits the induction. Lorenz *et al.* (87) and Kaplan *et al.* (88, 90 to 93) were apparently inclined towards the humoral view, and Furth & Upton (85) believed that the normal tissues may be capable of neutralizing a factor liberated from irradiated tissues. The nature of the interactions between irradiated and normal tissues and the influence of the endocrines on irradiated tissues remain obscure, but the existence of these physiologic interactions that influence leukemogenesis is unquestioned.

Cutaneous carcinogenesis has been explored further, using methylcholanthrene (MCA) and $\beta$ radiation singly and together (94). When both agents were applied externally, they were approximately additive, but when applied by injection of pellets, they were not completely additive. The authors suggest that the high doses of radiation destroyed the area of local MCA effects from the pellet. Production of ovarian tumors was a common effect of whole body radiation (WBR) of mice. In the $LAf_1$ mouse it was noted that WBR produced the most effect, irradiation of ovaries less effect, and WBR with ovaries shielded, the least effect. This would imply again that there are interactions between irradiated tissues prone to develop tumors and the remainder of the body (95). Chromophobic tumors of the adenohypophysis occurred spontaneously in aging $LAf_1$ mice. Radiation hastened the development and increased the incidence (96, 97, 98). In this case, it appears that the tumor-inducing action of radiation may be related to acceleration of a normal aging process, rather than directly to a carcinogenic process. Morphologically similar tumors have been seen after radiothyroidectomy (99, 100, 101).

Concepts of the mechanism of development of pituitary tumors in mice have varied. In the case of basophil adenomas, the explanation appears to have been fairly well established. The stimulus of castration plus the proper amount of WBR seemed to be adequate to explain the genesis of basophilic adenomas (99). However, the genesis of the chromophobe tumors has not been this simple. In the work of Upton *et al.* (98), chromophobe adenomas apparently developed spontaneously, and the incidence was increased and the time of onset was accelerated by WBR. In another work (99), it was

stated that Dent and Furth produced adenomas by surgical thyroidectomy alone. However, their animals were given a tracer dose of $I^{131}$ to test for the presence of thyroid tissue, which, in case of almost complete absence of thyroid tissue, may have given a significant dose of WBR. Edelmann (99) apparently believed that thyroid insufficiency with increased TSH secretion, plus the insult of WBR or partial body radiation (head or body), was sufficient to induce pituitary chromophobe adenomas in a strain of mouse that spontanesously developed few, if any, of these tumors. The fact that chromophobe adenomas may arise from different stimuli or spontaneously in different species and strains is quite evident. It appears that an understanding of the mechanism of pituitary tumorigenesis will not be attained easily until all investigators describe more fully the experimental procedures used in their induction and the morphologic and functional characteristics of these tumors.

When irradiated rats were protected from an otherwise absolutely fatal dose of WBR by parabiosis subsequent to exposure with a nonirradiated rat, or by pre-irradiation administration of glutathione or para-aminopropriophenone, they developed diverse types of tumors (102, 103). A high incidence of the tumors were of epithelial origin and metastasized widely. Lymphoma, the commonest tumor produced by radiation, was not seen during the observation period. A localized pencil of deuteron irradiation [Swift *et al.* (104)] produced ulceration and scarring, followed by development of an adenomatous, apparently nonmetastasizing tumor, which, in some cases, ruptured and produced "pseudomyxoma peritonei." Irradiation of the kidney produced hyperplasia of tubular epithelium similar to the renal carcinoma produced by WBR in parabiosis experiments. Again, one wonders if the WBR disturbs the interelationships of tissues so that benign tumors may become highly malignant, metastasizing, invasive tumors. Were this generally true, one might question the wisdom of radiation therapy of extensive areas of the body. The studies of Koletsky & Gustafson (105) on WBR as a carcinogenic agent (based on survivors from an $LD_{70/30}$-day dose of x-radiation) showed that a wide spectrum of carcinomata and sarcomata occurred earlier and with a greater incidence than in the control, nonirradiated rats. It appears from these several studies that WBR accelerated the development of an internal environment conducive to the development of diverse tumors, often multiple in the same animal. The presence of certain normal shielded tissues (ovaries, hemopoietic tissue) or the introduction of homologous cell suspensions (bone marrow) inhibited the development of specific tumors. Chronic irradiation of $LAf_1$ mice [0.11 r/day; see (106)] produced tumors similar to those obtained following single doses of radiation, but with an increased incidence.

### Physiological and Histological Effects

Because of space limitations, most of the papers listed in this section cannot be given the attention deserved. The review of Gray (107) on biological damage by ionizing radiation considered the fundamental interac-

tions among the cell, the cell's environment, and the energy deposited by radiation. Glucksmann (108) considered the relative levels of radiosensitivity of somatic cells, a subject of considerable importance to those concerned with radiotherapy and carcinogenesis. Extensive data on the effects of atomic bomb $\gamma$ radiation on the 30-day mortality rate in mice have been reported (20). The spontaneous course and features of radiation illness and morbid pathology in the monkey (109 to 112) and in the bat (113), after various times and doses of x-ray, have been presented. The histopathologic changes in hamsters after large supralethal doses of WBR were presented by Rugh *et al.* (114). The pattern of weight loss in the irradiated rat (115, 116, 117) and the relation of body weight to mortality and x-ray dose in the mouse (118) are now systematically presented. Tullis *et al.* (119, 120) and Gleiser (121, 122, 123) presented the mortality and pathology of radiation injury produced by WBR in swine and dogs, respectively. Rajewsky (124) continued his earlier studies on the effects of WBR in mice as a function of dose, dose rate, and time after irradiation. Ellinger *et al.* (125) have published on the reproducibility of the lethal effect of WBR in mice, showing the necessity of rigidly controlled radiologic and biologic factors.[8] Dowdy & Bennett (126) discussed WBR with reference to both acute and long-term effects. Reinhard *et al.* (127) emphasized the marked strain differences in response to partial body radiation in the mouse, particularly in regard to endocrine influences. Jones *et al.* (128) have studied volitional activity in the rat after WBR (200 to 1000 r) and found a significant depression. At the lower doses the depression was moderate and recovery rapid. With decedents which survived longer than nine days, there was an initial depression, followed by recovery and a subsequent depression that lasted until death. Splenectomy of infant and pubertal mice did not influence radiosensitivity to WBR (129). Blair continued his very interesting mathematical analyses of longevity in animals given internally deposited isotopes of Ra, Po, and Pu (130, 131) and of recovery rates after WBR as determined by mortality studies in survivors. His analyses lead to certain conclusions:

> While life shortening may be attributable, especially in some of those animals which attain a large fraction of their normal life span to various specific pathologies, there seems to be no doubt that, in addition to or underlying the identifiable causes of death, there exists in the irradiated animal a diffuse state of deterioration which, for lack of better expression, is termed premature ageing. The fact that all chronically irradiated animals, whether they exhibit specific pathologies or not, appear to conform to the same life-span dose relationship suggests that premature ageing is the principal or primary effect and that this gives rise prematurely to those specific pathologies which were destined to appear eventually. The alternative to such a view is that the life span is determined to so large a degree by the premature ageing that the occurrence of specific pathologies does not appreciably alter the outcome.

In addition, Blair concluded that radiation injury was proportional to the

[8] However, the most careful attention to experimental conditions does not obviate the absolute necessity for simultaneous exposure of experimental and control animals in any system of biological assay.

dose, and was partly reparable and partly irreparable. The reparable part subsided exponentially and the irreparable part accumulated in proportion to the dose. The animals' life span was shortened in proportion to the sum of the reparable and irreparable injuries at the time of death. Formulations of these hypotheses appear to represent essentially all of the data on the lethal effects of radiation in mammals, except those on the life shortening consequent to large, single, sublethal doses. These doses shorten the life span several times as much as do divided doses of the same magnitude. If these data apply to man, the hazards of single, large, sublethal doses to this generation is evident.

*Oral radiation deaths and effects.*—The cause of death attributable to WBR is known to vary with dose and time after radiation, and each cause of death presents a different pathologic picture. Deaths "under the beam" or shortly after exposure to massive doses (central nervous system deaths), deaths 3 to 6 days after exposure in the high-lethal range and above (gastrointestinal deaths), and deaths 7 to 20 days after exposure in the lethal range (hemopoietic deaths) are well reviewed (1 to 4, 9, 61). Quastler (132) has presented data showing that irradiation of only the head, jaw, or tongue of the mouse with 1500 r or more produced 8 to 12 day deaths with a steep dose-mortality curve. The deaths were not attributable to starvation or aplasia of the marrow; the cause of death has not been determined as yet. English *et al.* (133 to 136), in systematic studies on the effect of radiation on the oral structures, have made similar observations, as have Nemus *et al.* (137). Deaths at postirradiation times similar to those observed by Quastler have been observed after large doses of radiation in the monkey (138). Reinhard *et al.* (127) have presented data indicating a wide range of sensitivities of mice of different strains to selective irradiation of the head, as determined by acute mortality.

*Gastrointestinal.*—Earlier studies by one of the authors [Bond *et al.* (61)] have been extended by Farr & DeBruyn (139). In their studies, shielding of exteriorized rabbit intestine did not influence the survival rate. The dose level employed, however, was not sufficiently high to result in the early "G.I. death" produced by exposure of the abdomen, exteriorized bowel, or by high doses of WBR. (A survival time of 3 to 6 days was obtained, as compared with 11 days in the series of rabbits reported by Farr & DeBruyn.) Their conclusions, that external shielding of the abdomen results in a summation of protection from diverse intra-abdominal organs, appeared sound but did not eliminate the influence of the bowel on survival, which can be demonstrated only by irradiation of bowel alone with the rest of the body shielded. Maisin *et al.* (140), using various sized shields and multiple shields over the abdomen, have concluded that simultaneous shielding of portions of the bowel and bone marrow yields an increased protection by a synergistic action. The complex interrelationships of dose, body region or organ exposed or shielded, mortality rate, and survival time have been reviewed recently (61). Conard (141, 142) has studied the serial changes in weight and contents

of various portions of bowel of rats given WBR. Results demonstrated the marked functional disturbances of the bowel induced by radiation. In addition, he has developed a biological radiation dosimeter, employing loss in weight of the small intestine as the index. Swift *et al.* (143) studied the delayed gastric emptying time in rats induced by radiation exposure and demonstrated that the effect can be produced by selective irradiation of body regions remote from the stomach. (A marked effect was produced by head irradiation alone.) Intragastric irradiation of the dog has been studied (144). Death occurred in some animals but could apparently be prevented by early gastrectomy. Cytochemical alteration in DNA, RNA, and alkaline phosphatase of the epithelium of the G.I. tract of the rat have been studied by Tillotson & Warren (145). Fluctuation in content of DNA, RNA, and phosphatase was observed. Cells of the crypts of the small intestine were most sensitive and showed typical modification during the destructive and reparative stages. Chinn & Wang (146) have shown that bilateral destruction of the emetic chemoreceptor zone prevented radiation-induced vomiting. Since the above procedure and use of chlorpromazine (147) minimize vomiting, and shielding of the trigger zone while irradiating the rest of the body does not influence vomiting, it appears that radiation does not have a direct action on the zone but mediates the action through a chemical mediator such as acetylcholine. Other studies on G.I. function were recorded (148, 149).

*Hemorrhage and platelets.*—General concepts on the nature of radiation-induced hemorrhagic syndrome have been reviewed (150). Heparinemia is not the cause of bleeding, and the amount of available heparin may actually be reduced after WBR (151). Most *in vivo* and *in vitro* clotting defects were correlated with the degree and duration of the thrombocytopenia. However, there was some evidence that a plasma lipid antithromboplastin may have accounted for some *in vitro* discrepancies (152). The importance of the platelet has been recently emphasized by Lamerton *et al.* (153), who found that leg shielding lessened the severity of the thrombocytopenia and, concomitantly, the anemia. In fact, as long as the platelet levels remained above 40,000/mm.$^3$, practically no anemia developed; whereas, when levels below 30,000/mm.$^3$ were attained, a severe anemia develoved rapidly. This occurred at the time when manifestations of hemorrhage become apparent. White *et al.* (154) have confirmed the relations of thrombocytopenia, prolonged coagulation time, and deficient prothrombin utilization in the dog, and Trum & Rust (155) in the burro. White *et al.* (154) have also shown that soybean phosphatide corrected the defect in prothrombin utilization *in vitro*, and *in vivo* can shorten clotting time and improve prothrombin utilization without affecting the platelet count or clot retraction. Copley & Stefko (156, 157) studied hemostasis in sympathectomized and adrenalectomized animals before and after irradiation, and apparently no additional defects were detected other than that expected from each procedure alone. Savitsky *et al.* (158) reported alterations in clot retraction time and clot adhesiveness following WBR in the dog and asserted that eight days after

irradiation a substance which inhibited clot retraction time and decreased platelet adhesiveness was demonstrable in the plasma. In other unconfirmed experiments, Savitsky (159) claimed to control radiation hemorrhage with injections of a splenic extract that contained a clot retraction accelerator. The importance of confirmation and studies on effectiveness of this splenic factor in a wide spectrum of animals, for both fundamental and practical purposes, is evident. Vetter & Vinazzer (160), in an unconfirmed report, stated that there was a deficiency of proaccelerin in $P^{32}$-treated patients. Platelet count levels in human beings exposed to 175 r fallout radiation have been followed (39). Minimal values occurred 28 days after exposure. Recovery was slow and still not complete 12 months after exposure. Hemorrhage did not occur, although in some instances, platelets fell to approximately 50,000/mm.$^3$

*Leukocytes, lymph nodes, spleen, and bone marrow.*—Reports continued on the response of the peripheral blood to WBR in the monkey (109), in the hamster (161), and in man after fallout radiation (39). The late depression in the granulocytes, minimal at 45 days after a sublethal dose of radiation in man (175 r or less), was unexpected. Gershon-Cohen *et al.* (162) have studied the response of leukocytes in the rat to doses of 85 to 5000 r, and concentrated on counts made during the first 24 hr. postirradiation. The early prompt lymphopenia and neutrophilic leukocytosis were again observed. Williams (163) has analyzed the value of blood studies in protection against radiation injury and has concluded that numerous routine counts are unnecessary where good physical control of the environment was available, but that more highly controlled hematologic studies were indicated in known radiation areas in order to obtain more information on threshold and permissible values. Helde & Wahlberg (164, 165) quantitatively related the low-level WBR exposure of human beings to changes in the leukocyte counts and believe that the blood was a good indicator when counts were obtained at the appropriate time. Ackerman *et al.* (166) performed extensive cytochemical studies on the lymph node and spleen of rats at various times after exposure to 600 r WBR and described distinct differences in dying and surviving cells. Latta & Waggener (167) have systematically studied effects of 4 mc. of $P^{32}$/gm. rat on hemopoietic organs and peripheral blood. Smith & Holst (168) have made serial studies on histochemically detectable lipid in thymic cells after irradiation. Plasmacytoid cells were observed to increase in number in the lymph nodes, spleen, and bone marrow but not in the thymus of irradiated dogs (169). Human leukocyte levels during roentgen therapy have been studied (170). Lymphocyte, and, to a lesser extent, neutrophil levels correlated with integral dose. Eosinophil, basophil, and monocyte counts did not correlate with dose.

Thymic weight studies have been extended by Kallman & Kohn (171). The time of minimum weight and dose response did not follow a simple relationship. The data behave as if there were two independent cell populations with different sensitivities. However, thymic weight 5 days after ir-

radiation was a linear function of the logarithm of the radiation dose as reported earlier by Carter (see 52), and the system was useful as a biological dosimeter. Another careful mathematical analysis of the weight loss of thymus and spleen was that of Stroud *et al.* (172).

*Erythrocytes.*—Chanutin *et al.* (173) found that administration of phenylhydrazine prior to 500 r markedly increased rate of onset of anemia. Erythrocyte fragility and effect of trace dietary minerals in the diet of irradiated rats were studied (174). Long-term feeding of polycythemic cobalt diets increased the mortality and affected fragility to a lesser degree. Uptake of $Fe^{59}$ by red cells in ducks after 600 r was followed (174). The expected depression was found and ascribed to a decrease in the number of the youngest types of reticulocytes. Aplastic anemia has been observed in 4 survivors of the atomic bomb (175).

*Skin.*—Irradiation effects on the skin assume more practical importance today, since the beta skin burns from fallout radiation following large atomic bomb explosions have now been shown to be possible (39). Space limitations forbid adequate coverage of even the selected papers listed (176 to 193). Lushbaugh *et al.* (176 to 180) have reported extensive studies on the local histopathological and biochemical effects of beta burns in rabbit skins. Witten *et al.* (181, 182) have studied the relative effect of $\gamma$- and $\beta$ irradiation of human skin and described the effects of a pure $\beta$ emitter, $Sr^{90}$. Edgerley (183), studying rates of build-up and disappearance of bullae in skin produced by various agents, concluded that x-irradiation increases capillary permeability, observable 24 hr. after 500 r, by means of a direct effect on the ground substance. Upton & Gude (184, 185) confirmed the increased capillary permeability, but found it to occur at a later time than that noted above. The increased vascular permeability began five to seven days after irradiation and lasted from one to two weeks, coinciding with the hemorrhagic phase, and was characterized by increased amounts of intercellular ground substance and a diminished number of mast cells. Smith & Lewis (186, 187) and Pettersson (188) have independently reported dissolution of mast cells in the dermis. Decreased numbers of mast cells evident between 10 and 33 days after 600 r WBR in the hamster have been noted (187). Merwin & Hill (189) published data showing that local doses of x-ray to skin in excess of 500 r slowed that rate of vascularization of areas that had received thermal burns. Nödl (190) reported the influence of fast neutrons on human skin; Ungar & Damgaard (191) found an inhibition of inflammatory response by $\beta$ irradiation; Prodi & Miceli (192) studied the diffusion of fluids in skin after x-ray; and Gros, Mandel & Rodesch (193) reported on the influence of x-rays on phosphorus and adenosinetriphosphate (ATP) content of rat skin. From the accumulated studies, there can be litle doubt now that irradiation, in addition to causing epidermal injury, influences the dermis of the skin as well.

An interesting and useful phenomenon, the de-pigmentation of mouse hair following exposure, has been used as a biological radiation dosimeter

(29, 194). Detailed serial histological studies on the skin and hair follicle cycle of the mouse have been performed (195).

*Central nervous system.*—The nervous system generally has been considered radioresistant. However, the term is relative and it depends upon the biological endpoint studied. Hicks (196, 197), studying development of the nervous system, found that the embryonic brain is sensitive and that diverse abnormalities could be induced by relatively low doses of radiation. Langham *et al.* (198) have studied in great detail the clinical and biochemical behavior of monkeys and rats dying after high dose-rate, high dose radiation. Andrews & Brace (199), in studying terminal phenomena associated with massive doses of x-ray (25,000 r), described clinical and electroencephalographic (EEG) changes and concluded that the dysfunction appeared to be subcortical and not the direct result of ionization in the brain tissue. Further studies of EEG and neurological effects of radiation have followed (138, 200, 201). Of interest were the marked early neurological signs after doses in excess of 2000 r to the head (138). Arnold *et al.* (202, 203) concluded that the brains of man and monkeys are considerably more radioresponsive than is ordinarily supposed and that the effects were attributable to direct effect on the brain itself and not to vascular occlusion. The white matter of deep portions of the internal capsule and brainstem were more sensitive. Radiation paralysis resulting from cord irradiation has been reported by Brunst (204). It has been shown that clinical and histological effects on brain were not ascribable to changes in brain cholinesterase or blood glucose and that the incorporation of tracer phosphate into brain was not affected by 20,000 r in the mouse during life (205). The neurologic influence of 12,500 r to the brains of rabbits was reported by Gerstner *et al.* (206). The syndrome consisted of a prodromal period, followed by a convulsive, then somnolent, then ataxic phase up to death (mean survival of 2.5 days). In other studies, Gerstner *et al.* (207) determined that 8000 r to the head of guinea pigs depressed the pinna reflex within 3 hr. and abolished it within 24 hr. Alterations in function and structure of glial cells of primate brains have been shown to be dependent upon total dose, intensity, and time after irradiation (208).

*Chronic radiation.*—The risks from chronic low-level irradiation in man, and the control of such risks have been analyzed by Mole (209). It was concluded that blood counts allowed an evaluation of the degree of damage, but had little practical value in estimating the degree of harm likely to result from such damage. Only clinical experience can ascertain the degree of harm resulting from various injuries. In other studies, Mole (210) has emphasized the fact that in earlier studies of Lorenz *et al.* (reviewed in 1 to 4) on chronic irradiation, the pattern of dose and effect was drastically altered if one considered the "wasted" irradiation resulting from continuous irradiation to death. Daily exposure to radiation for limited periods of time can produce results quantitatively similar to those obtained by irradiation until death.

*Clinical features.*—The systematic, carefully controlled studies on radia-

tion sickness in man by Court Brown & Mahler (211, 212, 213) have significantly contributed to basic knowledge on radiation illness. From their correlation studies on body size, integral dose, clinical symptoms, and biochemical studies, they have shown that the latent period from the time of irradiation to onset of symptoms was dependent, within limits, upon integral dose and body surface area, and that the severity of illness was inversely related to the length of the latent period. From these studies, it was logically postulated that a diffusible metabolite is released by radiation and "The length of the latent period and the duration of the symptoms may be determined by the final concentrations of these agents in their volume of dilution, which may be the whole-body or some particular fluid compartment." This postulate gained added import, since Edelmann (214) has recently published evidence for the existence of a "toxic" metabolite in the blood of irradiated animals. In other clinical studies, Court Brown & Abbott (215) have shown that correlation of radiation sickness with dose can be made only by taking into account the known differences in radiosensitivity of different tissues, a conclusion which logically could be deduced from previous animal shielding studies [reviewed in (61)]. Cysteineamine was found ineffective in the therapy of radiation sickness (216). Robertson & Godwin (217) calculated on the basis of anatomical measurements the relation of dose received by the blood to that received by the bone marrow during $I^{131}$ therapy of thyroid diseases. The observations pointed out the difficulties in ascertaining the dose to critical tissues from internal emitters, and emphasized the necessity of careful hematological studies in order to avoid serious hematopoietic depression. Studies on the use of radiogold (218) showed that the usefulness of intravenous gold was limited, since neoplastic tissues of the RES did not pick up the colloidal gold particles. The hazards of colloidal radiogold (219) from intraperitoneal and intratumor injections have been demonstrated. Marked marrow hypoplasia was induced and the gold isotope was found in the marrow, showing that the material moved, despite its local deposition and colloidal state.

*Combined effects.*—The effects of combined injuries (radiation plus burns or wounds) has been studied (220 to 227). These studies have practical importance and are of general interest, since the effects were those of combined stresses. Burns plus radiation increased the mortality (222), apparently because of an increased incidence of infection. Irradiated animals apparently tolerated aseptic surgery well (226). The rate of closure of incised wounds performed under aseptic technique apparently was not impaired by 150 to 650 r (227). Air blast injury did not significantly affect the outcome of radiation injuries if animals survived the initial blast effects (225).

### Endocrines: Direct and Abscopal Effects

*Pituitary.*—Pituitaries of toads have been shown to be extremely resistant to irradiation [Allen *et al.* (228)], retaining their capacity to secrete thyreotrophic hormone at dose levels up to 20,000 r. Total destruction was not ac-

complished until a dose of 200,000 r was reached. The test system involved irradiation of metamorphosed toads and then measurement of growth after transplantation of the irradiated pituitaries into immature irradiated tadpoles. In contrast to this, 750 to 1100 r of WBR temporarily suppressed the secretion of gonadotrophins by rabbit pituitaries as measured by chick gonad response (229). Mateyko & Charipper (230) reviewed histological effects of radiation on the anterior pituitary and presented their own extensive data, showing marked histological effects of localized irradiation. Localized irradiations with $10^4$ rep of cathode rays, 8000 r (supralethal) with head shielded, and 1000 r (supralethal) WBR were used in their experiments. The doses employed were large, and conclusions relative to the sensitivity of the pituitary after doses in the acute 30-day lethal range cannot be drawn from the data. Reinhard *et al.* (127) felt that differing sensitivities to radiation of the pituitaries in the several strains of mice studied may account for the varying response obtained from selective head irradiation.

In later studies, Mateyko & Edelmann (231) detected decreases in gonadotrophins following WBR and irradiation of the hypophysis alone. Thyrotrophin and adrenocorticotrophin (ACTH) assays were also studied, and definite alterations were found. In general, pituitary hormone output, except for ACTH, was reduced. ACTH production concentration was increased promptly (1 hr.). The pituitary ACTH response was not modified by head shielding. Of interest, and not explicable, was that irradiation of 0.0069 $cm.^3$ of pituitary tissue by $10^4$ rep of 1100 KV cathode rays was lethal within 24 to 48 hr. Korson & Botkin (232), in continuing work on effects of WBR on pituitaries, found that 24 hr. after WBR basophilic granules were fewer, and acidophiles increased in number. These cytological observations were consistent with the general concept developed by most groups that irradiation induces an increased ACTH secretion at the expense of other pituitary hormones. Jacobsen *et al.* (233) showed that endometrial implants in the eye of the rabbit responsed with a surge of growth if irradiation of the rabbit included the pituitary region. Taber (234) reported that ACTH administration relieved the symptomatology of therapeutic radiation sickness and advised its use for WBR. The latter advice is questionable, however, since many reports covered in previous reviews have shown ACTH and cortisone to increase the mortality rate in total-body irradiated animals. Barlow & Sellers (235) found that the somatotrophic anterior pituitary hormone had no effect on survival, organ weights, or histological changes in irradiated rats; however, the initial loss of body weight was partially prevented by this therapy.

*Adrenal and adrenal hormones.*—In addition to effects on adrenal function, those features that might logically be produced by stimulation of the pituitary-adrenal system are considered here. Mole (236) coined a useful term, "abscopal," to describe the remote effects of radiation in animals, thus leaving the phrase, "indirect effects," for chemical effects mediated by products of irradiation of water. Nims & Sutton (237) presented data on the

rat which indicated that the polydipsia and decrease in adrenal cholesterol level following WBR were the result of increased activity of the pituitary-adrenal system and that the initial fall in liver glycogen was principally the result of lowered food intake. Pituitary adrenal activity following WBR prevented the fall of liver glycogen to levels that would otherwise be obtained with simple fasting. Lasser & Stenstrom (238), in a clinical study of patients following pelvic area irradiation, found the degree and time course of "radiation sickness" to correlate with changes in the absolute peripheral eosinophile count, but not with the Thorn ACTH-eosinophile, 4-hr. response test. They concluded that the adrenal cortex underwent definite changes in the course of irradiation, but that the changes probably were not related to clinical "radiation sickness." Santisteban *et al.* (239) showed that cortisone replacement therapy progressively restored the resistance of irradiated-adrenalectomized mice. However, events causing death in the irradiated-intact animals differed from those in the x-irradiated adrenalectomized group despite cortisone treatment, indicating that cortisone may only partially restore resistance. Bond *et al.* (240) obtained highly selective irradiation of various small portions of the rat with a "pencil" beam of 190 Mev deuterons, and found that the thymus, spleen, and adrenal weights characteristic of pituitary-adrenal stimulation resulted only if and when the irradiation given imposed severe stress on the animal, as indicated by the gross symptoms of illness and body weight loss. Such changes could not be elicited by irradiation of the adrenals alone, nor were they prevented by adrenal irradiation if additional radiation damage to other tissues sufficient to put the animal under "stress" were present. Raventos (241) attempted to separate the direct and abscopal components of the effect of total-body radiation on spleen weight in the mouse. If was not possible to do this from his data, because the spleen exteriorization procedure itself markedly affected spleen weights. Spleen weights were taken on the fifth post-irradiation day when recovery and extramedullary hemopoiesis would be marked, particularly in spleen-shielded animals receiving otherwise total-body radiation, but histological studies were not made. The isolated, perfused calf's adrenal gland was used to study the effects of $\gamma$ radiation on the secretion of adrenal cortical hormones (242). Secretion was markedly reduced; however, it should be noted that the doses used were in excess of 2000 r (some five times the $LD_{50}$ and always supralethal), and that the isolated organ was removed from secretory stimuli that may be present in the intact animal following acute total-body irradiation. Thus, the authors' conclusion that the adrenal cortex must be considered a radio-responsive tissue cannot be considered to characterize the role of the adrenal in acute total-body irradiation. The studies should be repeated, using adrenals irradiated *in vivo* after doses from which survival is possible, and later removed for perfusion studies. French *et al.* (243) have demonstrated early changes in plasma hydroxycorticosteroid levels, as well as changes in the peripheral neutrophile, lymphocyte, and eosinophile counts of monkeys given from 50 to 400 r total-body radiation.

The changes were maximal at 4 to 8 hr., and values were again normal by 12 hr. Shielding of the head or adrenals did not modify these early changes, implying that they were not the result of a direct effect on the pituitary-adrenal axis. Brayer *et al.* (244) found, after a supralethal dose of WBR (1000 r) in swine, that a marked increase in urinary excretion of total adrenal cortical steroids occurred, which was most pronounced in the first 24 hr. Wilhelm (245) studied adrenal hormone excretion in irradiated mice and found that corticoid and ketosteroid excretion increased on the first day with doses in excess of 100 r, and decreased with doses of 1000 r or more. Detailed histological studies on the lipids of the adrenal cortex (246, 247, 248) after various doses of irradiation (250 to 3000 r) indicated increasing activity with dose. These changes were consistent with published excretion studies, and with the concept of an abscopal effect. The studies, showing decreased ascorbic acid content of adrenals (249), were also consistent with excretion, organ weight, and histological studies.

*Thyroid.*—Cellular hypertrophy and morphologic nuclear changes in the thyroid are known to be produced by nonablating doses of $I^{131}$, appearing in one month and persisting for as long as 18 months. Since these changes are inhibited in part by hypophysectomy and DL-thyroxine injections, Maloof (250) concluded that these were secondary and not primary effects of irradiation. Monroe *et al.* (251) found that one day after appropriate doses of WBR to rats there was an increase in $I^{131}$ uptake not present at 7 to 30 days after exposure. Furthermore, the thyroids of burros that had been exposed to high levels of irradiation presented the histologic picture of hyperthyroidism. The histologic effects on normal and pathologic thyroids of various types of irradiation ($I^{131}$, implants, and external x-ray) have been well-described as a function of dose and time (252, 253, 254). Closon & Betz (255) have shown that WBR interfered with thyroid uptake of $I^{131}$ measured 5 days after 800 r. Betz (256) has studied the relationship of thyroid and adrenals after lethal irradiation. All observations appear to be consistent with those previously reviewed, i.e., that the pituitary secretion of ACTH increases at the expense of the other pituitary hormones. However, it is really too early to consider the problem settled.

*Gonads.*—Earlier reports on radiation damage to the testis have considered depletion of spermatogenic elements as being attributable to inhibition of mitosis in Type A spermatogonia. Oakberg & Crowell (257) and Oakberg (258) demonstrated in an entirely satisfactory manner that the reduction in spermatogonia resulted from killing of cells. His time studies depict the microscopically visible sequence of events. Injury to sperm and spermatids could be detected only by the resultant genetic damage after fertilization. Fogg & Cowing (259) correlated increasing and decreasing radio-resistance in spermatogonia with cytological appearance and reported infrequent necrosis of spermatogonia. However, Oakberg (258) criticized their work on the basis of confusion of spermatocytes with spermatogonia. Carter *et al.* (260) have studied the dose-rate, time relationships of sterility

induced by chronic irradiation of inbred CBA mice. An increase in sterility was induced by as little as 40 r at a rate of 1.64 r/week. The weight loss in testes of the mouse, rat, and hamster was studied as a function of x-ray dose and time after exposure, both for single doses at different dose rates (261), and for fractionated doses (262). Maximum weight loss occurred after 28 days in the mouse and 25 to 50 days in the rat and hamster. The weight loss has been expressed mathematically as a function of dose. In the fractionation experiments (262) the injury was proportionate to total dose and was not influenced by the fractionation schedule used. Rugh & Clugston (263), in studying sex differences and the relationship of the estrous cycle of the mouse to radiosensitivity, concluded that males are more sensitive than females at all stages of the cycle. They also showed that females are relatively resistant in estrus and sensitive in diestrus. These authors postulated that the hypersecretion of estrogens during estrus and its sequelae may account for the increased radioresistance during this phase of the cycle. However, the differences in survival, though consistent, were small.

Spellman *et al.* (264) confirmed an earlier report on the enhancement of mortality by testosterone propionate. The long-acting testosterone cyclopentylpropionate, injected every 14 days, improved the survival rate. The authors ascribed the apparent beneficial effects to a prolonged catabolic action.

### Embryology, Development, and Mammalian Genetics

The 1953 review of Rugh (265) is pertinent, and a symposium on effects of radiation on embryonic development has been published (266). The studies of Russell & Russell on mammalian embryology are of particular interest (267), as are their studies on mammalian genetics (see 63). Muller (268, 269) pointed out that the established permissible doses may result in radiation-induced genetic damage, a view which is more conservative than are those of most geneticists.

Greulich *et al.* (270) have studied the physical growth of children who survived the atomic bombing at Hiroshima and Nagasaki and found definite retardation of growth, which still persisted 5-1/2 yr. after the incident. Abnormalities in the pregnancies of women who were pregnant at the time of the atomic bombing and who manifested signs of radiation illness were studied by Yamazaki *et al.* (271).

### Biochemical Effects

An excellent analysis of chemical effects of irradiation on simple, complex, and living systems has been published in a series of 16 papers (272). Following an $LD_{50}$ dose of external radiation, potassium was lost and sodium and chloride gained by radiosensitive tissues (273). Serum potassium and sodium was not affected by 600 to 800 r x-ray in rabbits, except terminally when a high serum potassium might occur (274). Increases in serum iron following exposure to radiation, which were reported earlier (see other re-

views), have been confirmed (275). Plasma cholesterol and uric acid increased, while the AG ratio and cholesterolesters decreased during the first few days after 500 r WBR (276). Changes in ascorbic acid levels in adrenals and other tissues following total-body irradiation were reported (277). Changes in the adrenal ascorbic acid concentration were ascribable to pituitary activation. Aminoaciduria was folllowed carefully in a series of four human beings accidentally exposed to WBR in the laboratory. Significant increases in number and quantity of amino acids excreted occurred as early as 12 hr. and continued for 5 months after exposure. It was not possible to ascertain the exact cause, but possible mechanisms of aminoaciduria were discussed (278). Of considerable interest, and equally difficult to explain, were the changes in the respiratory gases of the irradiated burro (279). The RQ fell with accumulated dose delivered at the rate of 25 or 200 r/day. Since blood pyruvates were increased, there may have been a defect in the citric acid cycle. A dose dependent increase in tryptophan peroxidase-oxidase of rat liver after WBR occurred. It was an indirect response, however, since adrenalectomy prevented the increase (280). Bacq & Herve (281) have considered evidence for disturbances in coenzyme A metabolism following WBR. Studies have been reported that were interpreted by the investigators concerned to indicate no effect of WBR on coenzyme A (282, 283). No defect in benzoic acid metabolism of irradiated rats was found (284). Hydrogen peroxide-catalase interrelationships were studied in normal and irradiated rats (285), but no definite relation of catalase to changes in radiation mortality was ascertained. Levels of DPN and DPNH were measured in the livers of irradiated rats. No change occurred after 700 r and a moderate decrease occurred after 980 r (286). Deoxyribonuclease was measured in spleens of rats given WBR, and was found to be increased (287). Maximum increase occurred in 24 to 48 hr., and a maximal response was produced by 200 r. It was not possible to tell whether the change was a result of the changing cell population of the spleen or of a direct effect of the exposure.

Purine metabolizing enzymes have been studied (288). ATPase and 5-nucleotidase activity of hemopoietic tissues have been studied (289, 290, 291). Activities of both enzymes increased in the thymus and spleen of irradiated rats. The effects, within limits, were proportional to the dose of x-ray. Studies on the enzyme systems above, DNA content, citric acid synthesis, and histological appearance of locally irradiated spleens were carried out (290). Shielding of the body with irradiation of the spleen only prevented increases in the phosphatases, prevented inhibition of citrate synthesis, and lessened the histologic injury and decrease in DNA content. These workers concluded "that x-ray does not exert a direct effect on the enzymes involved in the biochemical reactions which were studied." However, since there is some evidence that hemopoietic cells of the spleen may rapidly interchange with other hemopoietic cells of the bone marrow (see section on restoration), their conclusions may not be correct, since the cells irradiated in the spleen may have been replaced by normal nonirradiated cells at the time of their study. Smith *et al.* (291) have correlated increased

adenosinetriphosphatase activity with involution, and decreased activity with hypertrophy; implying that adenosinetriphosphatase was more concentrated in the stroma, the portion of the spleen which, histologically, is relatively radioresistant. Harrington & Lavik (292) have studied DNA synthesis in the thymus after irradiation and found a differential effect on the various precursors of DNA: $P^{32}$, orotic acid, pyrimidines, and formate. Incorporation into DNA guanine was inhibited, but incorporation of adenine into DNA purines, and formate into DNA thymine and adenine were not inhibited significantly. Kowlessar *et al.* (293) found an increase in deoxyribonuclease activity in the urine promptly after irradiation and concluded that its source was not renal epithelium or blood cells, but probably deoxyribonucleases from tissues. The same group in later studies (294) found that deoxyribonuclease I and II are elevated in the plasma of irradiated rats. Maximum activity of deoxyribonuclease I was six days after exposure; and of DNAase II, one day after exposure.

Thomson *et al.* (295) found that WBR (800 r) diminished the concentration and turnover rate of DNA phosphorus in regenerating liver. The rate of incorporation of $P^{32}$ into cytoplasmic RNA of regenerating rat liver was shared equally by all of the subcellular cytoplasmic components.

White *et al.* (296), in studying dietary protein intake, food intake, and nitrogen excretion, concluded that increased nitrogen excretion was attributable to increased catabolism and that radiation, in addition to decreased food intake, increased the demand for endogenous sources of energy. As an outgrowth of their dietary studies on irradiated animals, they found that irradiated rats on a low protein diet developed cirrhosis, whereas those on a high protein or amino acid mixture diet did not (297).

Huang *et al.* (298) studied renal and hepatic function of rats given approximately an $LD_{50}$ of WBR. Glomerular filtration and tubular excretion remained essentially normal. Hepatic inactivation of antidiuretic hormone was not altered. Para-amino-hippuric acid uptake, and the $Q_{O_2}$ of kidney slices were decreased for 12 days post-irradiation, indicating a reduction in tubular function which returned to normal.

Cornatzer *et al.* (299) have studied the effect of WBR on liver, kidney, and spleen phospholipid synthesis. Synthesis in the spleen was reduced slightly. No changes were observed in renal phospholipid, or in cholesterol of the liver, kidney, or spleen. They also confirmed the previously reported rise in palsma cholesterol and phospholipid following total-body irradiation.

Entenman *et al.* (300), in continuing studies on blood lipids, found that plasma phospholipids increased after total-body irradiation in all species studied. The magnitude of change and the time of appearance, however, varied with the species. Serial studies in dogs showed that there were sharp increases several days prior to death.

## Resistance to Infection

Recent articles on the relation of radiation injury to resistance and infection quite adequately cover the field through 1954 (24, 61, 301, 302, 303).

The review by Talmage (303) carefully analyzes the relationship of radiation injury to the various defenses against infection. Defenses against infection can be divided into stationary and mobile forces. The stationary defenses are the surfaces of the body, the fixed phagocytic elements in the connective tissues, the connective tissues which react to local invasion, and the fixed macrophage elements in the various filters (spleen, liver, and lymph nodes). The mobile defenses are the circulating leukocytes and antibodies (natural and acquired) which can attack at the point of infection. Of great importance to the acquired antibody defenses is the ability to renew synthesis of specific acquired antibodies to which the host has been sensitized upon reintroduction of the antigen (anamnestic response).

Infection is well-established as one of the lethal sequelae of WBR in the lethal and supralethal ranges when the survival time is greater than three days. However, the complete absence of infection, obtained by irradiation of germ-free animals, did not prevent death (304). The survival time was prolonged, and the lethal dose was increased by a moderate amount. The systematic studies of Miller, Hammond & associates on the time of occurrence and duration of infection in irradiated mice have continued (305, 306). Infection has been shown to play less of a role in mortality in neutron-irradiated, as compared to x-irradiated mice (307, 308). Clapper *et al.* (309) have shown that irradiation shortens survival time when given three to six days before inocculation of mice with pneumococci. Vincent *et al.* (310) have studied the indigenous flora of the intestine of the rat and found that a lactobacillus is normally the most common organism. By seven days after 650 r, the pseudomonads increased in number and the lactobacilli decreased, so that the pseudomonads predominated and were found in the blood stream after invasion occurred. Hammond *et al.* (311), in independent studies along a comparable line, have shown that the normal mouse promptly disposed of pseudomonads that had been introduced into the G.I. tract, and the usual flora was re-established, whereas the irradiated mouse was unable to do so, and pseudomonads became the prevalent organism in the upper small intestine. Of considerable importance in understanding radiation infection are the studies of Gordon *et al.* (312) on normal and irradiated mice. *Serratia marcescens* were fed to mice and found to penetrate the normal intestinal wall, since mesenteric lymph nodes upon culture were found to contain this organism. In irradiated animals the organisms were able to pass through the lymph nodes and were cultured from the spleen.

Antibiotics have been shown to have some value in increasing the survival rate after doses of radiation in the lethal range (313 to 317), but were of no value after doses that were uniformly fatal. Coulter & Miller (317), in particular, have shown the value of using multiple antibiotics in succession. Their previous studies had yielded equivocal results when single antibiotics were used, but the use of penicillin followed by streptomycin produced impressive results. The relation of the granulocyte count to spontaneous infection in irradiated animals was reviewed in detail by Cronkite & Brecher (24). The

correlation of low granulocyte count with probability of death was good. However, the correlations did not of necessity prove a cause and effect relationship. In addition to the decrease in concentration of granulocytes in the peripheral blood, qualitative defects in the function of the granulocytes have been reported in a series of studies. Donaldson *et al.* (318), in studying phagocytosis of nucleated chicken erythrocytes by mouse peritoneal exudate phagocytes, found that there was no difference in the ability of phagocytes from normal and WBR (350 r) mice to engulf erythrocytes, but that a 350 r dose markedly impaired the ability of the phagocytes to digest the cells. Wilkinson (319) found that the ability of irradiated rat leukocytes to phagocytize plague bacilli was unimpaired for six days after 550 r WBR, and from the seventh to the thirteenth day it was depressed. The defect was considered primarily cellular, since normal plasma only partially corrected the defect.

Shechmeister & Fishman (320) have carefully studied the migratory ability of leukocytes from normal and irradiated rabbits. A phasic response was obtained after 500 to 800 r WBR with decreased migration on the third to fifth days and tenth to thirteenth days, and normal migration in the intervening period. The period of normal migration corresponded roughly to the abortive rise in granulocyte count. By the twenty-first day, complete recovery had occurred. One hundred roentgens of WBR did not influence the migratory rate of leukocytes. The decrease in migration was not attributable to leukopenia or to plasma factors. However, the influence on migration of the age of the leukocytes and of the changing ratio of mononuclears to granular elements was not studied. In further studies with WBR of rats, the phagocytic and bactericidal power of the blood was studied (321). Opsonic and phagocytic indices were increased at 2, 12, and 24 hr. after 600 r, and were below normal between the third and fifth days after irradiation. The bactericidal power of the blood was decreased three to six days after exposure. The decomplemented serum from irradiated rats was more bactericidal than was that for normal rats. Extracts of leukocytes from irradiated rats three days after irradiation showed no bactericidal activity against *Micrococcus aureus*, whereas extracts from leukocytes of normal rats one day after irradiation were actively bactericidal. The susceptibility of the irradiated rats to infection by *M. aureus* was correlated with the granulopenia and altered leukocyte functions described above. The criticisms of not determining the abilities of the different types of leukocytes separately in each test, and the influence of an ageing population resulting from the impaired production rates of leukocytes apply to this work (see 24). The evidence produced, however, unless proved otherwise, strongly indicates that there are functional abnormalities of the leukocytes in addition to the dose-dependent decrease in number. Taplin *et al.* (322) published data which indicated impaired ability of the phagocytic system of the RES to clear the plasma of prodigiosin colloidal dye after WBR (300 r or more) of the rabbit. These data are in contrast to earlier studies showing that there is no impairment with

other colloids such as colloidal gold (discussed in earlier reviews). Gabrielli and associates (323, 324) have shown that WBR and radiation from $CrP^{32}O_4$ induced definite changes in the phagocytic functions of the RES characterized by diminution in the rate of uptake and distortion of the distribution of the $CrP^{32}O_4$ in the hepatic lobules. In the case of the WBR, the abnormalities could result from nonspecific stress mediated through the pituitary adrenal axis or by direct radiation injury of the RE elements. Since localized irradiation of the RES by the $CrP^{32}O_4$ produced similar changes, Gabrielli and associates were inclined towards considering the RE system as highly radiosensitive. To answer the problem of relative radiosensitivity of the RES conclusively, further experimentation is indicated. Gordon, Cooper & Miller (325) have shown that the capabilities of rabbits to phagocytize living bacteria after WBR were unimpaired; however, after a few days the irradiated animals released the bacteria from the RE cells and a marked bacteremia resulted. The reasons the RES cannot kill the ingested bacteria are not known. The time of re-establishment of the bacteremia more or less parallels the depression in granulocytes and implies that there may be interrelationships between the circulating granulocytes and the power of the fixed macrophages to kill ingested bacteria.

The reduction in ability of rats to produce antibodies after 350 r of WBR was not influenced by somatotrophic (STH) hormone administered after irradiation (326). Nagareda (327) showed that irradiation of the hypophysectomized rat did not alter the impaired ability of the whole-body irradiated rat (500 r) to produce antibodies. Spleen shielding of the hypophysectomized rat during irradiation induced a return in ability to produce hemolysins to sheep cells. It was concluded that the pituitary was not essential for antibody production against sheep red cells in the rat. Smith & Gump (328) showed that no relation exists between survival and the ability to produce antibodies, as indicated by the degree of response of the rabbit to egg albumin determined prior to irradiation. Shechmeister and Bond had previously shown that irradiated mice were susceptible to avirulent plague bacilli. In continuing studies, Silverman *et al.* (329) showed that immunization of the mice against plague endotoxin, but not plague antibacterial antigen, eliminated the increased susceptibility to living avirulent *Pasteurella pestis*. They concluded that the increased mortality in animals injected with an avirulent *P. pestis* after sublethal irradiation was the consequence of an increased susceptibility to the plague endotoxin. Stoner & Hale (330) found that within two to seven days after exposure, mice irradiated with 650 rep became more susceptible to anaphylaxis by various antigen-antibody combinations. The animals were not more susceptible to histamine, however, although various antihistaminics afforded almost complete protection against fatal anaphylaxis. In further studies, Silverman & Chin (331) investigated the immune state of animals by actual challenge with tetanus toxin rather than by measurement of antibody levels by serological techniques. Inhibition of antibody production was found, the degree of inhibition and time to

recovery being proportional to the dose of radiation. Irradiation after the injection of antigen was as effective in inhibiting antibody protection as was irradiation before injection. The closer to the time of irradiation that the toxoid was administered, the greater was the inhibition of the immune response. Fulton & Mitchell (332) earlier presented evidence that irradiation neutralized an immunity to *Salmonella typhimurium* infection that had been induced prior to irradiation. These observations are not consistent with the findings of Silverman & Chin (331) nor with those of Paulissen & Shechmeister (333). The latter workers have demonstrated that immunity induced before irradiation will confer some protection on animals challenged with living bacteria after irradiation.

Harris *et al.* had shown previously that lymph node cells from the popliteal lymph node of the rabbit, which had previously been sensitized by injection of *Shigella dysenteriae* into the foot pad, would stimulate antibody production when injected intravenously into a normal rabbit. In their current investigations (334) they have studied the influence of WBR on the ability of these injected lymph node cells to induce antibody production in the irradiated rabbit. Presumably the injected cells contain antigen, or are actively producing antibody (or both), or have incorporated antigen into a first essential step for antibody production. WBR with 425 r 24 hr. prior to injection of the lymph node cells from the immunized rabbit did not influence the antibody response. In fact, the titers were higher in the irradiated than in the nonirradiated rabbits. Irradiation 1 hr. after transfer markedly reduced the antibody response. Irradiation 24 hr. after transfer partially reduced the response, and irradiation 48 hr. after transfer had no influence on antibody response of the recipient. In these studies, simple antigen was not injected. Presumably, the antigen was complexed with cellular constituents and, when injected into an irradiated animal, antibody production continued, whereas the antigen injected at this time produced virtually no antibody. The antigen-cellular complex was sensitive to radiation for a short period, however, but became radioresistant by 48 hr. after injection, at which time active antibody production continued. The mechanism of antibody production by the cell transfer technique is not well understood. In the irradiated rabbit, however, it appears that there is a radiosensitive and radioresistant phase.

Impairments in the bactericidal power of serum from irradiated animals have been demonstrated by Donaldson & Marcus (318, 335) and Shechmeister & associates (320, 321, 333, 336). Pillemer & associates (337) described a diminution in properdin[9] concentration of irradiated rat serum at the time when rats were susceptible to infection. Stroud *et al.* (338) have found protection with Fraction III (Cohn), and Pillemer *et al.* believed that their results may have been on the basis of properdin, since the latter is concentrated in Fraction III.

[9] Presumably a natural, nonspecific antibody, in the Landsteiner concept.

In summary, much progress has been made in understanding the causes of the increased susceptibility of WBR animals to infection and numerous factors are involved. Although in most species, infection is a dominant cause of death in the lethal range, obliteration of infection by the germ-free state does not eliminate death from other causes. Protection by the mucosa of the normal bowel is relative, since bacteria can permeate the normal bowel. The stationary defenses are altered. Changes in the morphological appearance and in the permeability of the connective tissues of skin following irradiation have been reported (see section on skin). The importance of these changes in defense against infection is not known. The ability of fixed RE cells to phagocytize bacteria appears to be relatively normal following WBR, but the ability to retain and kill ingested bacteria appears to be impaired. Granulocytes are diminished in number, and evidence that they are functionally deficient accumulates. New specific antibody production and the anamnestic response for acquired antibody production are drastically inhibited. Lastly, the titer of natural antibodies (properdin) appears to be reduced. Thus, infection would be expected as a common sequelae of irradiation. Elucidation of the possible mutual dependence of the RES granular leukocytes, and natural antibodies for normal function may be possible by means of leukocyte transfusion, with and without properdin.[10]

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[10] The authors are indebted to Marjorie Comstock, Brookhaven Research Library, for excellent bibliographic assistance.

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# COMPARATIVE PHYSIOLOGY (BEHAVIOR)[1,2]

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The years 1950 to 1955 have been a time of such intense research activity on a variety of problems in animal behavior that it will be possible to do no more than briefly indicate the status of several outstanding problems.

Several books of general interest have appeared during the period. The *Handbook of Experimental Psychology*, edited by Stevens (1), contains several useful chapters on comparative behavior studies and on the physiological correlates of various behavior patterns. *Insect Physiology*, edited by Roeder (2), contains a number of very up-to-date review chapters devoted to behavior and its physiological bases, particularly those by Schneirla (3 to 6) on insect behavior and social patterns, Roeder (7, 8) on nerve activity and organization, and Dethier (9, 10, 11) on sensory processes. Several books by Tinbergen (12, 13, 14) give a good picture of the point of view and activities of the flourishing European school of comparative behavior, as do several chapters in a Biological Symposia volume on *Physiological Mechanisms in Animal Behaviour* (15).

## THE ANALYSIS OF "INSTINCTIVE" BEHAVIOR

### The Neurology of Instinctive Behavior[3]

Among the most prominent influences on the study of animal behavior in recent years has been the work of Lorenz (16) and various others influenced by him, including, prominently, Tinbergen (12, 13, 14).

Lorenz deals with a group of movement patterns, involved in what are traditionally called "instinctive" activities, which have characteristics that are not readily explicable on the basis of traditional reflex, or chain-reflex, concepts. These movement patterns occur in the natural behavior patterns of the animals concerned and are of a type that has not been very much approached by traditional neurophysiological investigation. Examples are certain courtship movements in fishes, food-begging movements of young birds, the movements by which nesting geese roll their eggs back into the nest, movements used in display and fighting by birds, etc. Lorenz points out that such movements: (*a*) can be elicited by definable combinations of stimuli; (*b*) can be elicited more readily when they have not recently been performed; (*c*) can be elicited by less and less specific stimuli, the longer they have gone

[1] The survey of the literature pertaining to this review was completed in September, 1955.

[2] The following abbreviation is used in this review: CNS (central nervous system).

[3] The author is indebted to Dr. F. H. R. Prechtl for several references and for a discussion of his forthcoming paper (66).

unperformed; (*d*) appear to be very rigid in their form; (*e*) appear to be exhaustible relatively specifically; and (*f*) can occur spontaneously. The time relations involved in the exhaustion and recovery are so long as to preclude any significant role of the exhaustion of peripheral neural or motor elements.

Such phenomena as exhaustion and recovery of a centrally-organized pattern lend themselves very well to representation by hydraulic models, and Lorenz has developed such a model in great detail. He assumes that each "instinctive act" represents the outflow from a neural center of a reaction-specific energy which has accumulated in the center. He assumes, further, that each such center is normally held under inhibition by a second center, called an "innate releasing mechanism," which is inherently tuned to a specific set of stimuli which will remove the inhibition. A further assumption is that the movement is entirely centrally-coordinated and centrally-produced, peripheral influences playing no role except to trigger the outflow. A summary statement would be (16) " . . . some sort of energy, specific to one definite activity, is stored up while this activity remains quiescent, and is consumed in its discharge."

In the available space, it is impossible to convey the extraordinarily wide influence that this hydraulic model has had on the work of a great variety of students of animal behavior. It has been particularly useful as a conceptual model for various observers of the social behavior of animals in nature (e.g., 13). It is of great theoretical importance because it attempts to provide a physiological rationale for traditional concepts of intra-central organization, spontaneity and specific motivation, of instinctive acts.

Some recent work, however, suggests that, although the hydraulic model has been most useful in stimulating research and in arranging a great deal of data, it is becoming possible to deal with the type of behavior involved without assuming either independence of the movement from peripheral influences or central accumulation and exhaustion of reaction-specific energy and that the hydraulic model can give way to more neurologically-oriented types of explanation [Lehrman (70); Kennedy (71)].

*Afferent adaptation.*—Prechtl (17, 18) studied the food-begging ("gaping") responses of young songbirds to various stimuli. He found that to any one effective stimulus [e.g., the (imitated) call of the parent or the shaking of the nest] typical exhaustion and recovery data could be obtained. However, he then repeatedly elicited the response by an auditory stimulus, then did the same by shaking the substrate, then repeated the auditory stimulus, etc. Under these conditions, he found that exhaustion with respect to one stimulus leaves the animal relatively unaffected in its readiness to make the response to the other stimulus. This indicates that the exhaustion of the response is attributable in large part to some sort of afferent adaptation, rather than to exhaustion of a reaction-specific motor excitation. Prechtl further noted that hungry animals would respond more readily than nonhungry ones, but that the response would not be more intense, merely elicitable by a greater variety of stimuli. This again suggests changes in the afferent sys-

tem. It should be pointed out that the time relationships are such as to make it quite unlikely that the adaptation of receptor cells is the primary process.

Hinde (19, 20) studied the characteristics of the "mobbing" response of chaffinches, which is normally given to certain predatory birds and animals. By studying the elicitation of the response by various stimuli, and the waxing and waning of the response, he was able to show that the normal waning of the response upon repeated elicitation includes a component of habituation to the specific stimulus.

Grunt & Young (21) found that male guinea pigs will not copulate with the same female for some time after ejaculation, but that, if the female is replaced by another, recovery of readiness to copulate is very much faster. Part of the "exhaustion," therefore, is attributable to a stimulus-specific adaptation.

Franzisket (22), studying the "wiping reflex" of spinal frogs, also found stimulus-specific refractoriness under conditions of time-relations which precluded receptor adaptation. Further discussion of this point will be found in reviews by Hinde (23) and Lehrman (24).

Afferent, but not necessarily receptor, adaptation appears to have been demonstrated electrophysiologically by Hernández-Peón & Scherrer (25), who showed that spikes recorded from the cochlear nucleus in response to auditory clicks presented to the ear of cats at the rate of about 30 per min. would gradually die away, without, however, reducing the response to other auditory stimuli.

*CNS and afferent sensitivity.*—Another factor of some potential importance is the fact that changes in the CNS may cause changes in afferent activity and thus be partially involved in changes in responsiveness. Centrifugal impulses change the sensitivity of retinal [Granit (26)] and cochlear [Galambos (27)] receptors. Stimulation of various loci in the reticular formation specifically alters sensory thresholds of, for example, muscle spindles [Eldred (28)]. Reticular stimulation also causes changes in afferent volleys resulting from dorsal root stimulation [Hagbarth & Kerr (29); Hernández-Peón & Hagbarth (30)]. The importance of the reticular formation as an activating system which helps determine the level of responsiveness and of motivation of an animal has recently been pointed out by Hebb (31) and by Lindsley (32).

*Afferent effects on level of responsiveness.*—The question of whether the cessation of an instinctive activity is a result of the using up of specific, centrally-produced excitation has been considered in several ways. Moynihan (33) showed that in the Black-headed Gull, (*Larus ridibundus*, the tendency or drive to incubate is reduced, not by performing the act of sitting on the eggs, but by finding a full clutch of eggs there. Bastock, Morris & Moynihan (34) show, in a variety of cases, that cessation of an "instinctive" activity is attributable to the occurrence of an external stimulus situation into which the animal is brought as a result of the performance. It is probable that proprioceptive stimuli may play a similar role in many cases. It appears, for

example, that proprioceptive stimuli arising from the act of eating play a role in the satiation of hunger [Lorber, Komarov & Shay (35); Kohn (36); Berkun, Miller & Kessen (27)], as do interoceptive stimuli from the digestive tract [Hill *et al.* (38); Paintal (39, 40)].

*Afferent effects on central motor organization.*—It is well known that central motor structures, even those which yield definite movements upon direct stimulation, may vary in sensitivity and in organization according to the nature of the concurrent afferent inflow. Gellhorn and his collaborators (41, 42) have shown that a wide variety of afferent inflows can change the motor effect of stimulating a particular point on the motor cortex. Delgado (43) similarly showed that the ease with which he could elicit various movements by cortical stimulation depended upon what his freely-moving cats were doing. Kempinsky & Ward (44) cut the 8th cranial nerve and found decreased limb movement in response to cortical stimulation, an indication of how indirect central-peripheral relationships may sometimes be. Malis, Pribram & Kruger (45) found action potentials in the motor cortex upon stimulating peripheral nerves. In a series of papers Lassek (46, 47, 48) has shown that dorsal rhizotomy causes severe atonia and motor paralysis.

*Motor localization of instinctive behavior.*—The well-known work of Hess [summarized by Gloor (49)] has been cited as evidence that instinctive coordinations are localized in the hypothalamus and (12) that the hypothalamus is the highest center involved in "instinctive"behavior. However, Hess had found that the effect of stimulation of a hypothalamic point depended partly upon the nature of the afferent inflow, so that the "reservoir" of instinctive energy remains completely elusive. Further, many of these hypothalamic reactions are also elicitable from the cortex, indicating a much more complex organization than that implied by a model with centers functioning as reservoirs [MacLean & Delgado (50); MacLean (51); Kaada *et al.* (52, 53, 54); Hess & Akert (55)]. Further, "instinctive" behavior has been interfered with by various types of cortical ablation [Beach and collaborators (56, 57, 58); see also Hinde (59)].

*Spontaneity and endogeneity.*—An important point in considering the hydraulic model is whether spontaneity of behavior necessarily implies endogenous production of fully-formed acts in the central nervous system, as implied by a reservoir analogy. There is ample evidence that the nervous system is continuously active, even when not being stimulated from outside. Roeder (60, 61) marshalls a great deal of evidence for the occurrence of spontaneous activity in nerve elements and in nerve systems. However, except for one or two cases of respiratory movements, the evidence does not warrant the conclusion that spontaneous, endogenous outflows can organize coordinated behavior. The frequently-cited experiments of Weiss (62) show that coordination does not depend upon specific reflex relationships, but they do not demonstrate the endogenous production of coordinated behavior, either. Weiss showed that deplanted fragments of spinal cord would establish connections with similarly deplanted limbs and that spontaneous activity of the

limbs would occur, but this activity was not coordinated, but "epileptiform" or spasmodic [Weiss (63); Desmedt (64)]. Coordinated activity occurs only when the limb is in connection with part of the nervous system that has connections with the periphery and with the rest of the nervous system. As Roeder indicates, there is a substantial gap in our present knowledge of the relationship, if any, between spontaneous neurone activity and spontaneous behavior.

Gray (65) shows that coordinated locomotor movements take place in Amphibia only when at least one limb has an intact afferent nerve supply. However, the afferent inflow can be quite simple and unspecific, and the motor coordinations may still be quite complex and highly organized. Prechtl (66) in a thorough review of this subject, shows that such intra-central coordination depends upon a wide variety of factors, including afferent inflow, intra-central receptors, and collateral relationships among motor units.

*Conclusion.*—The phenomena which led to Lorenz's hydraulic model, and to his assumption of intra-central production of intra-centrally coordinated, intra-centrally motivated behavior, seem susceptible of analysis on a basis which does not require the erection of so many new neurological categories. The model, although it has been very useful in many ways, has probably now outlived that usefulness.

Most of the evidence cited so far has been from mammalian nervous systems. However, similar conclusions can be reached from much lower levels. Van der Kloot & Williams (67, 68, 69), in a remarkably penetrating analysis of the cocoon-construction behavior of the Cecropia silkworm, show that a variety of environmental factors, environmental changes brought about by spinning activity, internal changes brought about before and by spinning activity, and neural relationships co-operate in the organization of this behavior. Even at the level of this preeminently "instinctive" act of a preeminently and traditionally "instinctive" animal, spontaneous, organized activity does not necessarily imply an intra-central reservoir.

## Hormonal Influences on Behavior

Several excellent reviews of the role of hormones in behavior have been published, notably by Beach (72, 73) and Collias (74).

Koller (75) reports that nest-building in the white mouse can be increased either by progesterone injection or by giving the mice newborn young. The finding by Stone & King (76) that hypophysectomy increases nest-building in the rat suggests that a variety of mechanisms, rather than a unitary one, is involved. Ramsay made hens become broody by exposing them to chicks (77). The suggestion that the external situation changes the hormonal condition is in line with recent data on neural and "psychic" control over endocrine activity [cf. Beach (78) for review]. Huston & Nalbandov, for example, found that mechanical stimulation of the oviduct of a hen by an egg passing though it reflexly caused suppression of luteinizing hormone secretion by the pituitary (79).

The problem of the route by which hormones influence behavior has received some attention. Birch & Clark (80) showed that estrogen-induced changes in dominance level in female chimpanzees are probably attributable partly to changes in the genital and perianal skin which, as sources of stimulation, change the activity levels of the animals. Beach & Levinson (81, 82) find that changes induced in male sex behavior by androgen administration depend partly upon the development of papillae on the glans penis which increase the tactile sensitivity of the glans. Lehrman (83) showed that the effect of prolactin, which causes ring doves to feed squabs by regurgitation, is probably mediated by the hormone's effect on the crop of the bird. These data should not, however, lead to an oversimplified assumption that hormones always act through simple peripheral effects. Kaufman (84) showed that hormone-induced mating behavior in female rats did not depend upon the presence of the vagina.

The problems of individual differences in behavioral responsiveness to hormones, and of the role of experience, are just beginning to attract attention. Allee & Foreman (85) show that the extent to which androgen administration raises the social status of a hen in a small flock is different for different breeds of hen. An important series of papers from Young's laboratory is devoted to this problem. Grunt & Young (86, 87) showed that individual guinea pigs differ characteristically in their behavioral responsiveness to sex hormone administration. They measured the sex activity of guinea pigs, then castrated them. After castration, most of the animals dropped to a minimal level of sexual activity. Androgen administration about four months later resulted in the animals reaching the same level of sexual activity that had characterized them before castration, regardless of amount of hormone injected (above a specified threshold). Valenstein & Young (88) found that guinea pigs raised in social groups would develop higher levels of sexual activity than those raised in isolation. After castration, both dropped to the same minimal level. Androgen replacement therapy, however, caused the sexual activity level of each group to rise to the level that had characterized it before castration, indicating that the experience of the animal helps determine the type of effect that a hormone will have on its behavior. Schwartz & Beach (89) and Rosenblatt (90) found that the amount of sexual experience, in cats and dogs, affects the rate at which sexual behavior dies away after castration. Lehrman (83) found that prolactin would make ring doves feed squabs (by regurgitation) only if they had had previous breeding experience.

## BIRD NAVIGATION AND ORIENTATION

More work has been done, and more light been thrown, on the fascinating problem of the basis of homing and orientation in birds during the past five years than in any other period. These contributions center about the work of Kramer in Germany, Matthews in England, and Griffin in the United States,

all of whom have written useful summaries of the problems [Kramer (91); Matthews (92); Griffin (93, 94, 95)].

Griffin distinguishes three types of homing. The first type is that in which the bird depends upon visual features of the environment and explores randomly until it finds itself in a familiar locality. The second is a type in which the bird is able to orient itself in a particular direction, even in and through a strange locality. The third is that in which the bird is able to orient itself toward a home point from and through unfamiliar localities, regardless of the direction.

The first type of homing does not present any great theoretical difficulties. Griffin & Hock (96), captured a number of Gannets on their nests on an island in the Gulf of St. Lawrence. These birds are never seen away from salt water. They were released over 200 miles from their nests, at a locality over 100 miles from the nearest salt water, and were followed individually in a light airplane. The birds took off in random directions from the release point and followed random (or spiral) paths. About 62 per cent of them returned to their nests. It seems fairly clear that they had flown about in an exploratory manner until they came upon a familiar locality or at least to a shoreline. Griffin (97) followed homing pigeons that had been released in unfamiliar territory that had certain topographical features in common with the area in which they had been trained and found that they were disoriented, or misled, by the visual features of the environment. Wilkinson (98) and Griffin (95) have demonstrated that much homing data can be accounted for on the basis of such random search, combined with learned knowledge of the topography. Skinner (99) showed that pigeons could be trained to peck at one or another detail of a photograph, and could remember for very long times (up to four years) which part to peck at.

Some recent homing data, however, cannot be explained on such a basis. Terns released inland in New England tend to fly off rather consistently toward the southeast, regardless of whether this is the correct direction to their colony [Griffin & Goldsmith (100, 101)]. Clearly directional orientation has been shown in a number of cases. Several observers have observed pigeons until they disappeared from the release point and have shown clearly that they orient toward home while still in unfamiliar territory [Matthews (103); Kramer & St. Paul (104)].

Matthews (102) trained pigeons to home, with the training flights always in the same direction from home. Such birds, taken to a new and unfamiliar starting-point, would leave the release-point in the direction to which they had been trained, even though it was now incorrect. Further, this was observed at various times of day, so that no simple hypothesis of flying at a constant angle to the sun would suffice. Matthews (103) further observed that untrained pigeons released 50 miles from home would orient toward home (observations at the release point) but would not be successful in reaching home. This suggests that two separate processes are involved in the

homing: orientation from a distant point, which depends upon the bird's response to some physical definer of the position (geophysical or solar?) and which does not require learning of the topography; and finding the dovecote, which requires that the bird respond to learned visual features of the topography. Recent data by Matthews (92) strikingly confirm this. Watching the disappearance of trained pigeons from release-points that had not previously been used, he found that they were well oriented toward home from distances between 35 and 110 miles, and from distances of less than 10 miles. At distances of about 25 miles, however, they scattered randomly. He suggests that the 25-mile distance is great enough so that the pigeons were not familiar with the topography, and not great enough for the registration of a geophysical (or solar) difference between home and release points. The way to an explanation of these nontopographic homing and orientation abilities has probably been pointed out by a remarkable series of suggestions by Kramer and by Matthews.

Kramer (105) found that the characteristic restlessness of caged migratory birds during the migration season is not random, but directional. Using a closed cage with six equally-spaced windows through which the bird could see nothing but sky, he found that the orientation disappeared under cloudy conditions (106). He then placed shades and mirrors near the windows in such a way that the apparent direction of the sun's light was deflected. The direction of the bird's restless activity (*Zugunruhe*) was correspondingly deflected.

This perfectly clear demonstration that the bird's activity was oriented with respect to the sun has one quite remarkable aspect. The direction of the orientated activity does not change during the day, although the position of the sun, of course, does!

Kramer & St. Paul (107, 108) trained starlings to choose food from one of a number of food dispensers arranged in a circle, the training being to select that dish which was at a particular compass-point. After training, it was demonstrated that the choice of direction was based on the sun in the same way as in the case of "migratory restlessness." That is: the bird cannot choose correctly under cloud, mirrors can deflect the choice to the same degree that they deflect the direction of the sun's rays, and the bird trained at one time of day can choose the correct compass direction at another time of day.

Kramer (107, 110a) repeated this type of experiment indoors, using an artificial sun, the height of which could be adjusted to simulate various times of day. A bird was trained to select a given compass direction at a given time of day, with the artificial sun fixed at the appropriate height for that time. He was then tested at different times of day, with the "sun" remaining in its original position. The bird did not select the correct absolute compass direction. However, his choices were correct in the sense that the angle between his choice and the "sun" was the same as the angle between the true

sun and the correct compass direction at the time of day at which the test was carried out. In this situation, as in those previously described, the bird acted as if it were making allowance for the change in position of the sun at different times of day. This can only imply that the bird has some sort of diurnal metabolic cycle which can function as does a chronometer in human navigation, to give the time of day and, therefore, the relation of the sun's position to an absolute compass heading.

The factor of sun navigation is clearly important in homing. Matthews (109) released Manx shearwaters, a strictly pelagic species, inland, in an area where shearwaters are never seen. Under sunny conditions, the shearwaters homed to their nests at a rate which indicated quite direct flying. Under cloudy conditions, however, they did not home. Similarly, pigeons released in strange localities orient toward home while still within sight of the release point, but only in sunny conditions [Matthews (110)]. The accuracy of orientation in both these cases was independent of time of day.

To explain these facts, two alternative explanations have been offered. Matthews (110) believes that the bird observes the shape of the arc being described in the sky by the sun's path and, taking account of the time of day, extrapolates to obtain the highest point, which (in the northern hemisphere) is due south. The bird would have become familiar with the characteristics of the arc at various times of day at its home locality. Comparison of the highest point and of the arc angle between it and the sun's position with the corresponding data at the home locality can give the latitude and longitude change. Matthews does not suggest that the bird can make these calculations, but only that observation of the arc and integration of the observations with a "chronometer factor" and with memory of the arc (including time factors) at the home locality can provide a basis for the bird to alter its flight direction according to whether the arc looks too steep or too shallow, and thus keep on a home heading.

Such a theory requires that, when a bird orients toward home, it must have had sufficient time to observe the arc so that its shape could be compared with the corresponding shape of the home arc. Several observations indicate that pigeons may show orientation within 10 to 40 sec. after being released, which poses a great difficulty for a sun-arc theory [Pratt & Thouless (111), Kramer (112)], although Matthews has tried to show that such fine discriminations are theoretically possible for the bird's eye.

Kramer (110a) has suggested, alternatively, that the bird locates itself absolutely on some (unspecified) geophysical grid, but gets a home heading from the azimuth of the sun. That is, instead of the sun-arc giving the complete cue for the heading, as suggested by Matthews, Kramer believes that the bird "knows" that home is, say, to the east by reference to some nonsolar physical system of coordinates and that it uses the sun (with the "chronometer") to determine which way is east. The difficulty with such a suggestion is that all attempts to define such a grid in terms of physical forces

to which the bird might be sensitive have turned out to be quite unconvincing [for review see Griffin (94)].

Although both theories thus seem to require some doubtful assumptions, the work of Kramer and Matthews indicates clearly that sun-navigation and the ability to take account of the time of day play a crucial role in long-distance orientation.

*The chronometer factor.*—The reality of the bird's ability to react according to the time of day has been demonstrated by a number of experiments. Hoffmann (113, 114) trained two starlings to select food from a dish at the southernmost point of the perimeter of the experimental cage. He showed that the effects of this directional training lasted as long as $10\frac{1}{2}$ months. He then placed the birds in artificially-lit rooms in which the day-night cycle was 6 hr. later or earlier than the natural one. When the birds were tested under the natural sun after 12 or more days in the shifted cycle, the direction of their choices was shifted 90 degrees east or west, depending upon whether the cycle had been advanced or retarded.

Matthews (110) found that if he trained pigeons to home at only one time of day, and then tested them 6 hr. earlier or later, their orientations from the release point would be quite scattered, as compared with those of birds released at the training time. He found, further, that if he desynchronized the "chronometer" with the natural day by exposing the birds to irregular cycles of light and of feeding, their homing ability was severely disrupted. Matthews (115) also attempted to change the orientation of birds by Hoffmann's method of shifting the daily cycle and found that he could shift their orientation in homing experiments if he first disrupted the "chronometer" by irregular day-lengths, then placed the birds in a shifted daily cycle.

The importance of time-registration in the orientation of birds raises the question of the timing of biological "clocks" in general, on which considerable research is now being done. Rawson (116) kept two starlings in constant conditions of light, temperature, and sound and found that they maintained a daily activity cycle, but that it was not exactly 24 hr. Similar results were reported by Hoffmann (117) for a newly-hatched lizard. Aschoff and his collaborators have demonstrated diurnal activity cycles of approximately 24 hr. under constant light conditions in a variety of animals, including newly-hatched chicks (118, 119, 120). Such cycles, even when they occur in approximately 24-hr. rhythms under constant conditions of one or another environmental feature, become synchronized with the natural day through the effect of daily changes in the environment. For example, although Rawson observed cycles of slightly less than 24 hr. duration under constant light conditions, the cycle could be very readily shifted by imposing a new light-dark cycle. Browman (121) raised rats for 25 generations in continuous light and found that they still had a 24-hr. activity cycle, crudely measured in terms of activity during successive 8-hr. periods. He could shift this to a 16-hr. cycle by imposing changes in light and temperature.

The origin of such rhythms is quite obscure. Very few of the experimenters have kept animals in conditions which were constant for more than one or two factors (Rawson's is apparently an exception), so that endogeneity is very difficult to demonstrate from most of the available data. Brown and his collaborators have shown remarkably persistent activity rhythms in various invertebrates, correlated both with daily and with tidal periods (e.g., 122). He is not certain whether such rhythms are completely endogenous or partly timed by factors such as alterations in gravitational forces.

Some biological "clocks" regulating behavior may be quite independent from external timing for much of the time but require such exogenous imposition of a rhythm at some early time in development. Harker (123) found that mayfly nymphs showed diurnal rhythms of activity that were independent of environmental light, but that depended for their development upon the eggs or early nymphs having been earlier exposed to the environmental light rhythm. Pittendrigh (124) showed that the time of pupation of *Drosophila* is subject to a 24-hr. rhythm. Cultures raised in continuous dark show no such rhythm, but one can be imposed by a single experience of a dark-to-light transition.

## TAXONOMIC AND COMPARATIVE STUDIES OF BEHAVIOR

An important development of recent years, both for students of behavior and those of evolution and systematics, has been the increasing appreciation of the specificity with which behavior patterns characterize taxonomic groups at all levels. A good deal of earlier work tending in this direction has been synthesized by Lorenz (16) and Tinbergen (12). Several recent studies illustrate the value of behavior patterns as taxonomic characters. Marshall (125, 126), in a review of the bower-birds (Ptilonorhynchidae), shows that the bower-building behavior is a valuable taxonomic character demonstrating relationships within the family. He suggests that the cat-birds (Ailuroedidae) belong to a separate family, largely on the basis of the stage of development of their bower-building behavior, which is quite different from that of the true bower-birds.

Barber (127) separated a number of new species of firefly largely on the basis of differences in the characteristics of the flashes emitted by animals belonging to different populations. Fulton (128) in a study of field crickets found, within what had hitherto been considered one species, four different populations which differed with respect to various ecological and behavioral characteristics and which would not interbreed in the laboratory. These appear to be four good species in an early stage of divergence. Evans (129) provides evidence, on behavioral grounds, for the specific distinction between two species of spider wasp (Pompilidae) that are extremely similar morphologically. Evans also provides a very full and useful review of taxonomic studies of behavior (129, 130).

Cotter (140) recently analyzed the serological relationships among a variety of species of ducks and found that they confirmed changes that Delacour & Mayr (141), basing themselves on behavioral criteria, had previously made in the traditional systematic arrangement of the group. This is a striking demonstration of the validity of behavioral criteria in taxonomy.

A number of authors, especially Spieth, have provided systematic reviews of the behavior patterns of *Drosophila* species, which provide confirmation and some revision of the accepted taxonomy of the genus (131, 132, 133).

There are an increasing number of studies which, while not taxonomic in the strict sense, are comparative studies of the behavior of related species. Among others, there are papers on titmice [Hinde (134)], tylopode mammals [Pilters (135)], pipefishes and sea-horses [Fiedler (136)], pigeons [Goodwin (137)], mantids [Crane (138)], fiddler crabs [Peters (139)], and many others.

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